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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).22151 U.S. PTO  
60/569370

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
Trent Russell Neal Walter		Norten Woodbury		Tempe, Arizona Tempe, Arizona	
Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
LIGHT DIRECTED SOLID PHASE SYNTHESIS ON PATTERNED POLYMERS					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages 54		<input type="checkbox"/> CD(s), Number			
<input type="checkbox"/> Drawing(s) Number of Sheets		<input type="checkbox"/> Other (specify) Cover sheet; Postcard			
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.					FILING FEE AMOUNT (\$)  \$80
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Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME Robert D. Atkins

TELEPHONE (602) 229-5311

Date 05/6/04

REGISTRATION NO.  
(if appropriate)  
Docket Number:

34,288

112624.00138

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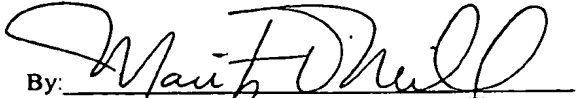
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**CERTIFICATE OF MAILING PURSUANT TO 37 C.F.R. 1.10**

Applicant: *Northen et al.*

Filed: May 6, 2004

Title: *LIGHT DIRECTED SOLID PHASE  
SYNTHESIS ON PATTERNED POLYMERS*

Docket No.: 112624.00138

Commissioner for Patents  
P.O. Box 1450  
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**PATENT**

**PROVISIONAL APPLICATION**

**of**

**TRENT RUSSELL NORTEN  
NEAL WALTER WOODBURY**

**For**

**UNITED STATES LETTERS PATENT**

**on**

**LIGHT DIRECTED SOLID PHASE SYNTHESIS ON PATTERNED POLYMERS**

**Attorneys:**

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PHOENIX, AZ 85004-2391**

**Express Mail Label No.: EL988555347US  
Attorney Docket No.: 112624.00138**

(Include additional names and addresses on a separate sheet.)

II. DESCRIPTIVE TITLE OF INVENTION

Light directed solid phase synthesis on patterned polymers

III. GRANT/CONTACT (If any):

Sponsor(s) none Award Number \_\_\_\_\_

Principal Investigator: n/a ORSPA Acct Number \_\_\_\_\_

IV. LAB/DEPARTMENT WHERE DEVELOPED:

Woodbury lab, Department of Chemistry and Biochemistry, ASU

V. DESCRIPTION OF INVENTION:

A. This invention is a(n): x process \_\_\_\_\_ chemical compound

\_\_\_\_\_ electronic circuit \_\_\_\_\_ mixture of chemical compounds

\_\_\_\_\_ apparatus \_\_\_\_\_ therapeutic method \_\_\_\_\_ other

(describe)

B. State, as fully as possible, what the invention is, including: materials or components used; operative and preferred ranges of process parameters and concentrations of chemical compounds; and foreseeable uses of the invention.

This invention combines three existing technologies: 1. photopolymers (photoresist), 2. photolabile protective groups, and 3. solid phase synthesis. The combination of these three existing technologies allows for the construction of three dimensional surfaces and devices that have tailored chemical functionality in spatially defined areas. The substrate can be any polymer that is in the appropriate three dimensional form having groups that can be derivatized in a way that can then be protected with photolabile protective groups. Photolabile protective groups can

three dimensional form having groups that can be derivatized in a way that can then be protected with photolabile protective groups. Photolabile protective groups can include any group that can be removed with light or activated by light in a way to expose or react with a material introduced in solution. Ways of patterning the polymer may include photopolymerization, thermal polymerization, or contact stamping. Ways of removing the photoprotective group include using a scanning laser system, micromirror array, or photolithographic method. Compounds that can be attached to this surface can be almost anything that will react with the given functionality exposed upon removal of the photolabile protective groups. It is possible to use both single and multiphoton excitation of the polymer and protective group to generate the spatial features. Sequential steps of removing the photolabile protective group and coupling new materials with the protective group blocking the appropriate reactive groups, can be used to generate complex patterns of functionalized polymer surfaces. In the short term these would be useful for enhanced DNA and Peptide microarrays, longer term these could be used for things as diverse as drug delivery systems, sensors, and artificial organs.

This includes a vast number of materials and methods. Work to date has been with acrylates and methacrylates including ones with reactive side chains (epoxy) that can be functionalized with diamines to yield aminated surfaces. These polymer surfaces have been three dimensionally patterned from photoreactive monomer/polymer solutions using a scanning laser system on top of a methacrylate functionalized glass surface. They have been protected with the nitroveratryloxycarbonyl (NVOC) photolabile protecting group (there are several protecting groups for amines and hydroxyls based on the general class of nitrobenzene compounds, though there are other classes of protective groups that can be used). This protective group has been removed using a scanning laser system. Detection of the deprotective areas has been done using fluorescence from a reactive dye that selectively reacts with the exposed amine groups.

- C. Records Supporting Invention: Identify records which establish dates of conception and reduction to practice, including identity of person who prepared record and its present location. Attach copies if possible. Note additional supporting evidence. If the invention or a significant aspect of the invention is not supported by written records, briefly describe how the date of invention can be established and identify earliest written record.

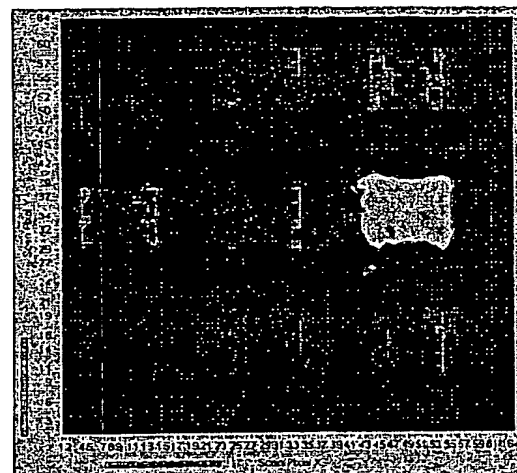
A glass cover slide was cleaned for 15 min at RT with 60/40 sulfuric acid/hydrogen peroxide, placed in 10% sodium hydroxide at 70 C for 3 min, placed in 1% HCl at RT for 1 min, between each step it was soaked in nanopure water for 3 minutes. A solution of 1% 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5 % water was made and mixed for 10 minutes, the slide was then added and left to react at RT for 15 minutes with gentle agitation. This slide was soaked in isopropyl alcohol for 3 min then nanopure water for 1 min then placed in a 100 C oven for 5 minutes after which the oven was

turned off and nitrogen was blown through for 1 hr. The slide was stored under nitrogen until it was used.

A blend of methacrylate monomers and photoinitiator (900 uL trimethylolpropane trimethacrylate, 100uL glycidyl methacrylate, 10 mg azobisisobutyronitrile) was prepared and nitrogen was bubbled through for 15 minutes before it was injected into a Focht cell (Biopetechs inc. Butler, PA) that had been flushed with argon. This cell was then mounted onto a Prior scientific microscope stage on a Nikon microscope and illuminated through a 40x 0.75 NA objective with 370nm light from a Ti:Sapphire laser modulated by a Conoptics modulator (shutter). The system was controlled via National instruments board and in house software. A pattern of nine square 500 micron features was patterned with 20 micron spacing at 99% scan rate with 100 microwatts of power input into the microscope ~ 25 microwatts output from objective.

Unpolymerized monomer was removed by washing with diethylether. The chamber was then filled with a 10 % 1,4-Bis(3-aminopropoxy)butane solution in dimethylformamide (DMF) for 15 minutes at room temperature. The chamber was rinse with DMF and then a solution of 14mg NVOC, 10 uL diisopropylethylamine (DIPEA), and 500 uL DMF was added and allowed to react for 35 minutes. The system was again rinsed with DMF and filled with dioxane. Two squares were scanned on the same laser system described above, with 10 scan lines per feature at 10% scan rate, one was scanned with 5 mW input power and the other 1 mW input power.

The chamber was then filled with a solution of 10mg dansyl chloride, 10 uL DIPEA, and 500 uL DMF and allowed to react for 15 minutes. The chamber was then rinsed with DMF to remove the excess dye and imaged on the same scanning laser system, where the 40x objective had been replaced by a 10x 0.30 NA objective. Fluorescence was collected via an avalanche photodiode which was processed by a Becker and Hickl Time-Correlated single photon counting module.



The data shows that the feature patterned with 5 mW (white) is significantly brighter than the other features (black/gray):

D. Fill in the following dates:

1. Conception Early March

2. First disclosure to another In early to mid-march I told a lab tech and undergraduate who work with/for me of the concept and told them that I was going to be focused on it from that point forward

3. First written record  
03/11/2004

4. First experiment demonstrating the invention  
04/14/2004

E. This invention can be used as Enhanced microarray technology where the signal is orders of magnitude larger making it easy to detect binding events (less sensitive instrumentation). Immediate application of this would be for DNA microarrays. This could also be used for the synthesis of large arrays of heteropolymers that could be used in drug development, molecular evolution, or sensor development. Hence, Lab on a chip analytical applications, analytical devices, or microsensor applications. Using multiphoton detection three dimensional surfaces can be decorated with functional groups using this method, these could be cell recognition factors, allowing the construction of complex three dimensional cellular arrays which could be used as bioreactors or artificial organs. These three dimensional surfaces could also be functionalized to form novel biomaterials or drug delivery systems.

F. The problem which this invention solves is  
Low signal from microarrays which very sensitive equipment to detect, inability to construct three dimensionally dimensionally functionalized materials with a high level of spatial control. Difficulty in screening and or encoding combinatorial libraries.

G. The closest prior art is  
Frechet Jean M.J. et al, Journal of Polymer Science Part A, 2002, Vol 40, 755-769 and Macromolecules 2003, 36, 1677-1684, used photolithography to prepare monolithic polymers in a spatially defined manner in glass capillaries.

Fodor et al, Science, vol 251, 767-773. Used photolithography in combination with a nitroveratryloxycarbonyl (NVOC) photolabile protective group to synthesize arrays of peptides on a glass substrate.

Satoshi Kawata et al, Nature 2001 vol 412 page 697-698. has created submicron objects using photopolymers in conjunction with two photon excitation.  
Shoji Maruo et al, Sensors and Actuators A, vol 100, 70-76. Has used single photon excitation to create 430nm photopolymer features.

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H. This invention differs from the closest prior art in that

Frechet used a very different chemistry. He uses free radical polymerization developed by (Ranby, B.) to graft polymers onto polymer surfaces. Therefore he can 'grow' a polymer on the surface of another polymer in a two dimensionally defined way. However, he does not have precise control on the products and can not synthesize heteropolymers with defined sequence in a spatially defined way. Where we propose spatially defined step wise synthesis; protect, deprotect, couple protected monomer/polymer, repeat until a complex structure is created. His method does not have the possibility of doing three dimensional controlled surface functionalization.

Fodor uses glass rather than a patterned polymer surfaces, he does use the photolabile protective in a repeated cycle of coupling and deprotection steps. He is limited to two dimensional patterning.

Kawata and Maruo have constructed three dimensional polymer devices using scanning microscopes but have not, to our knowledge done and synthesis or functionalization on the polymer surfaces.

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I. This invention provides the following advantages:

The ability to construct spatially defined functionalized polymer structures of great diversity in two and three dimensions. The larger surface area of the polymer vs. glass increases the signal and potentially the sensitivity vs. conventional microarrays.

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## **Patent Disclosure**

04/16/2004

### **Inventors:**

Trent Russell Northen, Neal Walter Woodbury, Sudhir Gudala.  
Department of Chemistry and Biochemistry, Arizona State University

Date of invention: 03/11/2004

Date of proof of principle: 04/14/2004

**Title: Light directed solid phase synthesis on patterned photopolymers.**

### **Overview:**

A new technology has been developed by the inventors that has potential to offer significant advantages over existing microarray technology with long term applications to sensor development, drug development, drug delivery, molecular evolution, and biomaterials.

This invention hinges on the combination of three existing technologies: 1. photopolymers (photoresist – plastic materials that can be patterned in three dimensions with light), 2. photolabile protective groups (these enable the patterned formation of specific chemical bonds in three dimensional space), and 3. solid phase synthesis (the process of generating complex heteropolymers such as, but not limited to, DNA and protein with known sequences on solid surfaces in a completely automated fashion). The combination of these three existing technologies allows for the construction of three dimensional arrays and devices that have tailored chemical functionality. Because of the three dimensional aspect of the photopatterning, an increase of several orders of magnitude in signal strength from, for example, DNA can be obtained as well as dramatic increases in the array density (both two and three dimensional arrays). It has the potential to change the paradigm for the current technology of both DNA and peptide arrays. In the long term this technology could be used to make sensors, combinatorial chemistry platforms, drug delivery devices, biomaterials, and even serve as the scaffold for generating artificial organs.

### **Huge Opportunity:**

"The total biochip market size in 2001 is about \$740 million and may more than triple in revenues, to about \$2.47 billion in 2006" (Technology, Strategic Alliance, Patent Dispute and Market Update – 2002).

Briefly, light is used to make little arrays of solid phase synthesis polymer (which is basically the same thing as photoresist used in the electronics industry) on a glass substrate. This polymer can be made in such a way that it is either porous or has a very rough surface (very large surface area) that is covered with reactive chemical groups (for example, primary amines). The reactive groups are then made unreactive by adding a

special blocking group that is photolabile (can be removed by exposure to light). Now, individual polymer elements of the array can be illuminated making them reactive in a patterned fashion and then reacted with materials of interest. One can then add specific molecules only to the elements that have been illuminated. If the molecules added themselves have reactive groups that are blocked by a photolabile blocking group, the process can be continued in layers, building up specific heteropolymers in a patterned fashion. The photolabile blocking group chemistry is the same as what has been used by Affymetrix and other companies to make DNA arrays. The difference is that instead of a monolayer of DNA (or peptide or other heteropolymer) on a surface, one has a much larger number of molecules in the same 2-dimensional element because of the third dimension afforded by basing the array on porous or rough-surfaced polymer elements. This greatly amplifies the signal, making it much easier to detect (the fluorescence from dye reacted directly with the polymer elements is easy to see by eye).

**Time Imperative:**

There is a group at Berkeley that has recently (2003) published very relevant work. That work does not yet include combining patterning of chemicals on polymer elements using photolabile blocking groups, but they have all of the technology available to them if they were to decide to go in this direction. It is critical that we move quickly to secure our rights to this potentially very significant invention.

Patent discloser:

Inventors: Trent Northen, Sudhir Gudala & Neal Woodbury

Date: 04.16.2004

Title: Light directed solid phase synthesis on patterned photopolymers.

Summary: A general method has been developed to create polymer features and modifying the functionality of the polymer in a spatially resolved manner using photolabile protecting groups to control the addition of desired functional groups.

This invention combines several existing technologies in a novel and useful way. The relevant technologies include: Solid phase synthesis, light directed polymerization, and light directed polymer synthesis.

Disclosed is the method of making polymer structures that have spatially defined chemical features through 1.) photopolymerization to form polymer structures 2.) protection of functional polymer features with photolabile protective group(s) 3.) photodeprotection of desired polymer features, 4.) reaction of deprotected reactive sites with desired reactive chemical species, and 5.) if desired repetition of these steps to form complex functional features.

The features of the polymer and photodeprotected region can be controlled through the modulation of the irradiating light. Small features (~1 micrometer) are generated using high numerical aperture objective lenses and even smaller features can be made using multiphase excitation (50-1000nm) or classical masking methods used in the semiconductor industry.

#### Background:

##### Photopolymers:

Photopolymer photo resists are well known and have been used for many years to create small features in the microelectronics industry. More recently they have been used in rapid prototyping or stereo lithography:

- Jan F. Rabek Mechanisms of photophysical processes and photochemical reactions in polymers 1987 John Wiley and Sons Ltd.

Most recently photopolymers have been used in conjunction with high numerical aperture lenses and multiphoton excitation to create very small three dimensional objects.

- Satoshi Kawata and coworkers, *Advanced Materials* 2003 vol 15, 2011-2014 has used single and multi photon interferential patterning to generate features as small as 50 nm.
- Satoshi Kawata et al, *Nature* 2001 vol 412 page 697-698. has created submicron objects using photopolymers in conjunction with two photon excitation.

- Shoji Maruo et al, Sensors and Actuators A vol 100, 70-76. Has used single photon excitation to create 430nm photopolymer features.

Spatially resolved biopolymer synthesis is well known and has been used for years to synthesize DNA arrays on glass substrates:

- Fodor et al, Science, vol 251, 767-773. Used photolithography in combination with a nitroveratryloxycarbonyl (NVOC) photolabile protective group to synthesize arrays of peptides on a glass substrate.
- McGall et al, JACS, 1997 vol. 119 page 5081-5090. Used photolithography in combination with the 5'-(( $\alpha$ -methyl-2-nitropiperonyloxy)carbonyl) (MeNPOC) to synthesize DNA arrays on glass substrates.
- Michael R. Sussman and co workers, Nature Biotechnology, vol 117, 974-978 used micromirror arrays in conjunction with the MeNPOC protective group to synthesize DNA microarrays.
- Gerard Cagney and coworkers, Nature Biotechnology, vol 18, 2000, 393-397 discusses different applications of protein and peptide arrays.

Solid Phase Synthesis (SPS) is well known and is a method of choice for synthesizing biopolymers (peptides, DNA, etc):

- Merrifield R.B., JACS 1963 Vol 85, 2149-2154 first synthesized a tetrapeptide on a solid resin particle (polystyrene).
- Barany G. et al, JACS 1996, vol 118, 7083-7093 has synthesized a solid phase resin that swells in both water and organic solvents using various methacrylate resins.
- Frechet Jean M.J. et al, Journal of Polymer Science Part A, 2002, Vol 40, 755-769 and Macromolecules 2003, 36, 1677-1684, used photolithography to prepare monolithic polymers in a spatially defined manner in glass capillaries.

Solid phase synthesis techniques have been used to generate combinatorial libraries. These methods have become common to the art, they typically include, dividing the SPS beads into pools after each synthesis step to generate large libraries of peptides. The peptide can be screened and cleaved from the bead can be encoded with some sort of tag for identification

- Lam Kit S. Chem. Reviews 1997, 411-448 this "One-Bead-One-Compound" method.

Photolabile protecting groups:

- Bochet Christain G., Journal of the chemical society, Perkin Transactions 1 2002 vol 2 125-142. Reviews the most common photolabile protective groups.

Biomaterials:

- Langer R. et al, Nature, vol 428 2004 487-492. Reviews biomaterial technology.
- Fisher J.P. et al Annu. Rev. Mater. Res. Vol 31 2001 171-181 describes photoinitiated polymerization and polymer crosslinking for biomaterial synthesis.

One of the significant disadvantages to the existing methods for spatially resolved biopolymer synthesis (Fodor, McGall, and Sussman) is the limited number of reactive sites available on the glass surface (McGall estimates 10-30 picomole/sq-cm). Characterization of reaction products becomes very difficult, requiring sensitive techniques and instruments, for example the most common technique, which is well known to one skilled in the art, for using and characterizing DNA arrays the hybridization of fluorescence probes and use of a scanning epifluorescent microscope to detect these probes. In the case of DNA since a fluorescently labeled complimentary strand can be made for each array element, it would in theory, be possible to characterize any DNA microarray with this technique under the appropriate hybridization conditions.

Since peptides cannot be probed in this same way, due to the non-complimentarity of their structures, other more complicated systems are used. Most commonly, the use of antibody systems in which one antibody is labeled with a fluorescent dye and one antibody (could be the same) is specific for the peptide sequence to be probed (Fodor). This is useful for a proof of principle, but would be impractical for probing large number of peptides.

Even though techniques have evolved to allow the synthesis and screening of libraries using SPS techniques (SPS) screening of the beads is complex.

Polymer structures can be functionalized using this method with cell recognition factors or binding factors such as.....these can be added/deprotected in a spatially controlled manner to create tailored structures. This invention combines the benefits of the array format, large number of reactive sites available in porous solid phase synthesis resin and the ability to form polymer structures using photopolymers. Resulting in larger signals, improved contrast ratios, and better applicability of analytical characterization techniques than existing microarray methods. Since the array is positionally encoded, it is easier to screen and probe than the split pool methods.

These are simply a result of having larger number of sites then on the glass substrate. So that the fluorescence signal is larger when using fluorescent probes or the amount of product produced in a given is large enough to be able to characterize products cleaved off the resin by common analytical techniques such as mass spectroscopy, FTIR, etc.

Further, the array format spatially encodes the peptides so that it is easier to probe than the split pool libraries. These arrays can be probed with analyte for sensor development, drug discovery, or for cell adhesion in biomaterial development.

This invention allows the generation of small three dimensional structures that can be functionalized in spatially defined ways for the construction of sensors, catalysis, biomaterials, drug delivery, molecular evolution, etc.

Summary of the invention:

The system is composed of a photopolymer bearing a reactive group, photolabile protecting group(s), groups to be attached that can also contain the photolabile protective group(s), and devices for illuminating the sample and introducing/removing new reagents. Groups to be attached are not limited to single molecules but could also include macromolecules and even cells.

#### Polymers/monomers:

For a system where it is desired to detect fluorescence from the array it is important that the polymer system not absorb the excitation light and that it not emit at the detection wavelength. In this case any nonfluorescent nonabsorbing (at the deprotection wavelength) and nonemitting (at detection wavelength) polymer or monomer systems can be used including monomers which are polymerized or polymers that are crosslinked or both. One or more of the following: acrylate, methacrylate, urethane, epoxy, urea, cellulose monomers, protein, glycols, lactic acid,  $\epsilon$ -caprolactone, trimethylene carbonate, N-vinylpyrrolidinone, 2,2 dimethoxy-2-phenylacetophenone, esters, DNA, RNA, .....containing side chains.....  
. or polymers of these monomers and or combinations of these monomers.

Solvents can be incorporated into these systems to modify the pore structure of the polymers. Solvents can include alcohols (methanol, ethanol, butanol, isopropanol, cyclohexanol), acetone, acetonitrile, toluene, etc.

Most preferred are methacrylates and acrylates.

#### Functionalization:

Polymers/monomers can themselves contain pendent reactive groups like hydroxyls, epoxy, amino, etc groups or they can be incorporated after the polymerization reaction.

#### Photoinitiators:

Photoinitiators (adapted from JP Fouassier progress in organic coatings vol 47, 2003 16-36) can include in the general classes of initiators: halogens, halogenated organic compounds, hydrogen peroxide, alkyl hydroperoxides, cumene hydroperoxide, peroxides, benzoyl peroxide, non-ketonic peresters, ketones, quinones, polycyclic hydrocarbons, azocompounds, hydrazones, cyclic acetals, 1,3-dithiolane, saccharides, metal oxides, ion pair complexes, metal chlorides, uranium salts, metal carbonyls, metal acetylacetonates, ferrocene, metal complexes, dyes, and polymeric photoinitiators. More specifically radical initiators: azides like azobisisobutyronitrile and derivatives, ketones like benzophenone, thioxanthone, acridone aromatic diketones and derivatives, ketocoumarins and coumarins derivatives; dyes (e.g. xanthene dyes such as eosin (EO) or Rose Bengal (RB), thioxanthene dyes or cyanins); thioxanthenes; bis-acylphosphine oxides; peresters; pyrylium and thiopyrylium salts in the presence of additives such as a perester; cationic dyes containing a borate anion; dyes/bis-imidazole derivatives/thiols; PS/chlorotriazine/additives; metallocene derivatives (such as titanocenes); dyes or ketones/metallocene derivatives/amines; cyanine dyes in the presence of additives;

dyes/bis-imidazoles; miscellaneous systems such as phenoxazones, quinolinones, phthalocyanines, squaraines, squarylium containing azulenes, novel fluorone visible light PIs, benzopyranones, rhodamines, riboflavines, RB peroxybenzoate, PISs with good photosensitivity to the near IR, camphorquinone/peroxides, pyrromethane dye, crystal violet/benzofuranone derivatives, two color sensitive systems, etc.

Colored cationic PIs (such as iron arene salts, novel aromatic sulfonium or iodonium salts) and PS/cationic PI (where PS can be hydrocarbons or ketones or metal complexes) can help to shift the absorption in the visible wavelength range.

Non-ionic photoacids and photobases for the generation of active species in photoresists technology are developed. By now, the design of colored species and proposals of PS for their decomposition remains attractive challenges.

Excited state processes of photosensitive systems for laser beams and/or conventional light sources induced polymerization reactions have been reported in recent works. Typical photosensitive systems under visible lights are classified as One-component system (such as bis-acylphosphine oxides, iron arene salts, peresters, organic borates, titanocenes, iminosulfonates, oxime esters, etc. Two-component system (working, e.g. through electron transfer/proton transfer, energy transfer, photoinduced bond cleavage via electron transfer reaction, electron transfer), Three-component system (where the basic idea is to try to enhance the photosensitivity by a judicious combination of several components).

Most preferred are Azoisobutyronitrile and its derivatives.

#### Photolabile protecting groups:

Photolabile protecting agents (from Bochet) can include: *o*-Nitrobenzyl alcohol derivatives,  $\alpha$ -Ketoester derivatives, Benzophenone reduction, Photosolvolysis-related reactions, Benzyl alcohol derivatives, Benzoin esters, Phenacyl esters, Acylating agents, Fluorene-carboxylates, Arylamines as photo-reductors, Benzophenone as photooxidant, Photoisomerisation *trans-cis*, Cinnamyl esters, Vinylsilanes substituted. Most preferred are nitroveratryloxycarbonyl, 5'-(( $\alpha$ -methyl-2-nitropiperonyloxy)carbonyl)

#### Groups to be added:

Groups to be added onto the polymer structures include, sugars, amino acids, nucleic acids, multifunctional amines, ethylene glycol, acid labile groups, base labile groups, dyes, .....and combinations of or polymers of these monomers. Sequential light directed synthesis can be used to build complex sequence specific polymers.

Most preferred groups include amino and hydroxyl groups.

#### Method of light modulation:

Light can be modulated (spatially patterned) using a scanning laser system composed of a laser, shutter, microscope objective and stage. In this case the stage movement and

shutter are controlled so that the shutter is only open when the stage is positioned so that the light will illuminate a desired position.

Photolithography is well known to the art but briefly it utilizes masks where light is blocked by some parts of the mask and not others. In this way the illumination reaching the sample can be controlled. Light sources typically include lamps or lasers.

Micromirror arrays are a more recent way of modulating light. By changing the angle of the mirrors in the array light can be directed towards a surface or not. In this way light from an excitation source (lamp or laser) can be selectively reflected onto desired regions of the sample to be exposed.

The preferred embodiment is either a micromirror array or scanning laser system

Substrate: Substrates can include glass, quartz, silicon oxide or other metal oxide surfaces, polymers bearing reactive groups. It is not necessary that they be transparent since illumination can be from above. In the case of glass, quartz, and silicon oxide these surfaces can be modified to react with the polymer for a covalent linkage, though this may not be desirable or necessary in all cases since intermolecular attractive forces can be used to 'glue' the features to the substrate. Where modification is desirable silanes common to the art can be used, the most common being aminopropyl triethoxysilane or 3-(trimethoxysilyl)propyl methacrylate.

The preferred embodiment is glass cleaned with acid and base as described in McGall JACS 1997 and functionalized from a 1% solution of 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5% water.

System for introducing reagents: Systems for introducing and removing reagents include an optical flow cell coupled with manual or automated introduction and removal of reagents. Wells or plates where reagents are introduced manually or by automation. Automation is provided by machines such as peptide synthesizers that are designed to introduce and remove reagents.

#### Analytical Techniques:

Array elements can be probed in situ through various spectroscopic techniques including fluorescence, absorption, infrared spectroscopy, raman spectroscopy, nonlinear spectroscopy, and surface plasmon resonance or elements can be removed from the surface through the use of labile linkages between the coupled material and the polymer. Thus the material can be cleaved and a host of analytical techniques can be used including HPLC, NMR, Mass spectrometry, capillary electrophoresis.

Most preferred include fluorescence detection of hybridized, bound, or covalently linked probes or groups, infrared spectroscopy, and mass spectroscopy of cleaved materials.

#### Example 1:

A glass cover slide was cleaned for 15 min at RT with 60/40 sulfuric acid/hydrogen peroxide, placed in 10% sodium hydroxide at 70 C for 3 min, placed in 1% HCl at RT for 1 min, between each step it was soaked in nanopure water for 3 minutes. A solution of 1% 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5 % water was made and mixed for 10 minutes, the slide was then added and left to react at RT for 15 minutes with gentle agitation. This slide was soaked in isopropyl alcohol for 3 min then nanopure water for 1 min then placed in a 100 C oven for 5 minutes after which the oven was turned off and nitrogen was blown through for 1 hr. The slide was stored under nitrogen until it was used.

A blend of methacrylate monomers and photoinitiator (900 uL trimethylolpropane trimethacrylate, 100uL glycidyl methacrylate, 10 mg azobisisobutyronitrile) was prepared and nitrogen was bubbled through for 15 minutes before it was injected into a Focht cell (Bioprotech inc. Butler, PA) that had been flushed with argon. The above slide was mounted in the flow cell. This cell was then mounted onto a Prior scientific microscope stage on a Nikon microscope and illuminated through a 40x 0.75 NA objective with 370nm light from a Ti:Sapphire laser modulated by a Conoptics modulator (shutter). The system was controlled via National instruments board and in house software. A pattern of nine square 500 micron features was patterned with 20 micron resolution at 99% scan rate with 100 microwatts of power input into the microscope ~ 25 microwatts output from objective.

Unpolymerized monomer was removed by washing with diethylether. The chamber was then filled with a 10 % 1,4-Bis(3-aminopropoxy)butane solution in dimethylformamide (DMF) for 15 minutes at room temperature. The chamber was rinsed with DMF and then a solution of 14mg NVOC, 10 uL diisopropylethylamine (DIPEA), and 500 uL DMF was added and allowed to react for 35 minutes. The system was again rinsed with DMF and filled with dioxane. Two squares were scanned on the same laser system described above, with 10 scan lines per feature at 10% scan rate, one was scanned with 5 mW input power and the other 1 mW input power.

The chamber was then filled with a solution of 10mg dansyl chloride, 10 uL DIPEA, and 500 uL DMF and allowed to react for 15 minutes. The chamber was then rinsed with DMF to remove the excess dye and imaged on the same scanning laser system, where the 40x objective had been replaced by a 10x 0.30 NA objective. Fluorescence was collected via an avalanche photodiode which was processed by a Becker and Hickl Time-Correlated single photon counting module.

The data shows that the feature patterned with 5 mW is significantly brighter than the other features (see image below):

## Example #2

A monomer mixture of the following composition was prepared: 1 mL hydroxyethyl methacrylate 2.6 mL Trimethylol propane trimethacrylate and 36 mg azobisisobutyronitrile. They were then sonicated for 5 minutes. Nitrogen was bubbled through the sample for 5 minutes. A biopetecs FSC2 chamber was purged with argon and then filled with the nitrogen flushed monomer mixture, with coverslip functionalized with trimethoxysilyl propyl methacrylate as described in example #2. The chamber was mounted on modified Prior scientific Proscan stage attached to a Nikon microscope. Laser excitation was obtained from a Spectra-Physics Tsunami mode-locked Ti:sapphire laser (742nm), which went through a Conoptics shutter and was later doubled to 371nm. This then was focused through a Nikon 0.30 NA 10x objective onto the sample. Laser power was set to 250 microwatts going into the laser with approximately half of that power at the sample. The photopolymer was patterned using in-house software, designed to control the stage and shutter. The following patterns were made:

Position	Power into laser ( $\mu$ W)	Exposure time (ms)	Number of features	Feature spacing ( $\mu$ m)	Focus vs. cover glass ( $\mu$ m)
1	500	500	5x5	1000	250 below
2	250	1000	5x5	1000	250 below
3	250	1000	5x5	1000	250 above
4	250	250	10x10	250	250 above
5	250	250	10x10	500	250 above
6	250	250	20x20	250	250 above

After patterning the chamber was rinsed several times with dimethyl formamide (DMF) to remove unpolymerized monomer. The hydroxyl groups of the polymer were coupled to a NVOC protected glycine (NVOC-gly). This was prepared from the amino acid and NVOC-acid chloride using schotten bauman procedure. The NVOC-Gly was activated with the coupling agent O-Benzotriazole-N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU) using the following procedure: 21 mg NVOC, 24 mg HBTU and 1 mL DMF were mixed and allowed to react for 30 seconds and 12.4  $\mu$ L Diisopropylethylamine (DIPEA) was added, this mixture was allowed to react for 3 min before adding to the chamber. This mixture was allowed to react in the chamber for 30 minutes without mixing and then another 30min with recirculation.

Any unreacted sites were acylated with acetic anhydride: a solution of 5 mL DMF, 146  $\mu$ L DIPEA, 100  $\mu$ L acetic anhydride was prepared. The chamber was flushed with DMF and then filled with this solution and allowed to react for 30 minutes. The chamber was then flushed with DMF and then filled with dioxane.

Features were deprotected using the same apparatus used to pattern the photopolymer. The laser was set to the same wavelength and the power was set to 500 $\mu$ W. For the larger patterns (1, 3, and 4) by manually finding the feature to be deprotected using the microscope and opening the shutter for ~ 30 seconds to expose the feature. The smaller

patterns (5 and 6) were deprotected by scanning adjacent features with the laser beam in a series of parallel lines forming squares. Position #5 was scanned with six 700  $\mu\text{m}$  squares with 700  $\mu\text{m}$  spacing between squares and each square was composed of 30 scan lines scanned at 1% of the maximum scan rate and 1 mW power into the microscope. Position #6 was scanned with 312  $\mu\text{m}$  squares with 448  $\mu\text{m}$  spacing with 20 scan lines at 1% of the maximum scan rate and with 1 mW power into the microscope.

After scanning the dioxane was drained and the chamber was flushed with DMF. The chamber was then filled with 10 mg/mL fluorescein isothiocyanate (FITC). This was allowed to react for 15 minutes. The chamber was flushed with DMF and sat overnight filled with DMF. The chamber was then flushed with fresh DMF and imaged.

Imaging was performed using the same scanning laser apparatus and laser configuration as used for the patterning and deprotection of the polymer-NVOC-GLY. The laser was set to 8  $\mu\text{W}$  input power into the microscope. Emission from the FITC was collected by an APD detector. Imaging was done with in-house software using a Becker & Hickl GmbH SPC-830 high performance photon counting board. All images were 64x64 pixels with the stage moving at 10% of maximum scan rate.

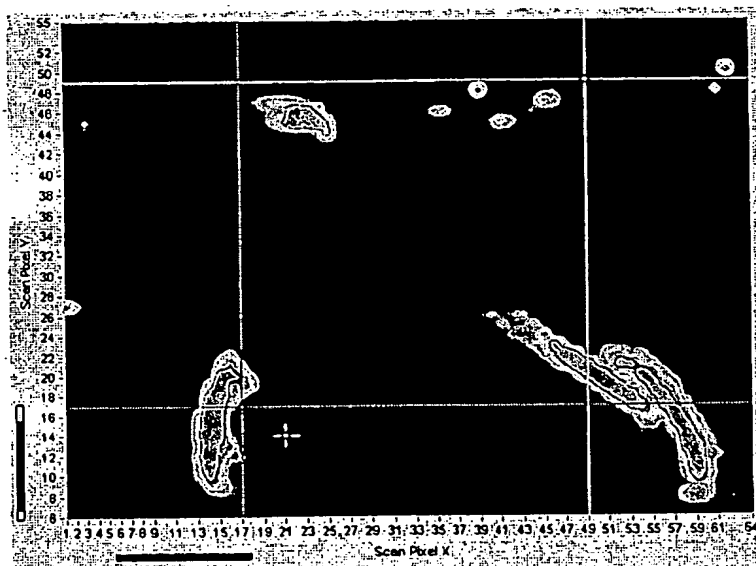


Image 1: Position 1 Shows the ordered spacing of the features (dark spots are unpatterned bright spots and lines have FITC. Note that the polymer is long hair like structures, some of which have fallen over.

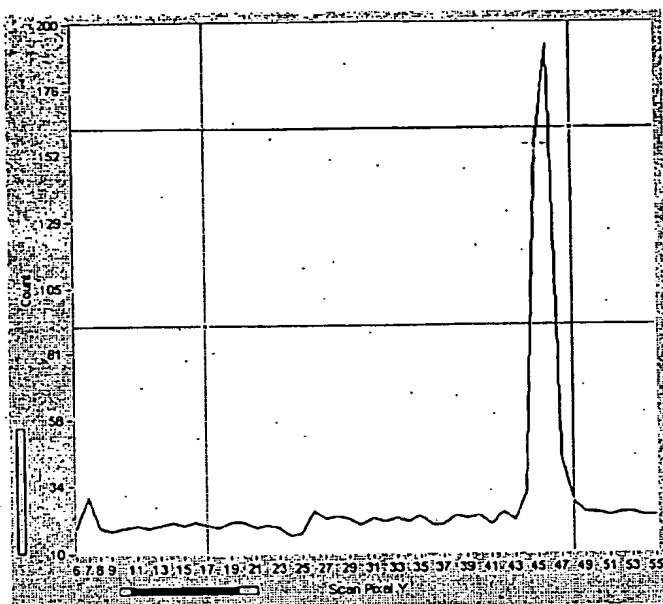


Image 2: Intensity x cross-section at scan pixel 23 of position 1. Note that the dip at  $y=24$  corresponds to a unpatterned feature and the peak at  $y = 46$  corresponds to a patterned feature. The image scan spacing was  $50 \mu\text{m}$  so the two features are  $\sim 1\text{mm}$  apart which corresponds to the distance between features in pattern 1. The contrast ratio is very high since the unpatterned feature is darker than the background.

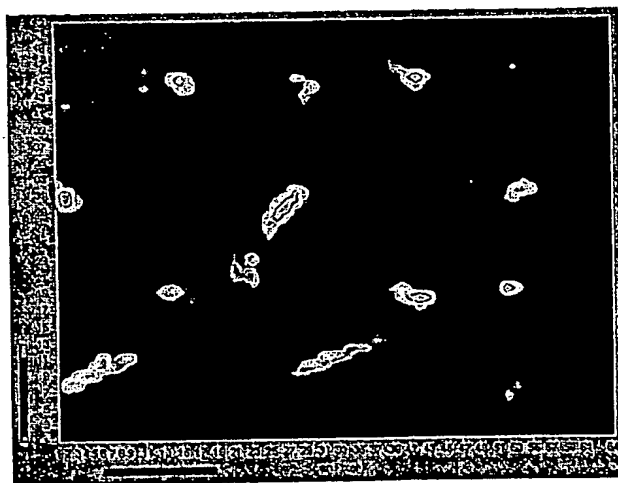


Image 3: Fluorescence intensity from position 3 shows alternating features of protected (dark) and deprotected (yellow, green, pink).

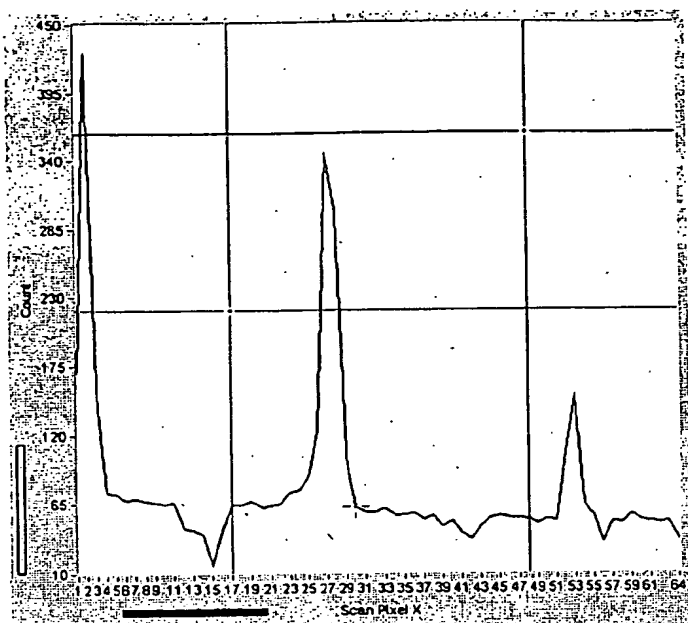


Image 4: Intensity x cross-section at scan pixel 30 of position 3. Note that the dip at  $x=15$  corresponds to a unpatterned feature and the peak at  $x = 2$  corresponds to a patterned feature. The image scan spacing was  $75\text{ }\mu\text{m}$  so the two features are  $\sim 1\text{mm}$  apart which corresponds to the distance between features in pattern 1. The contrast ratio is very high since the unpatterned feature is darker than the background.

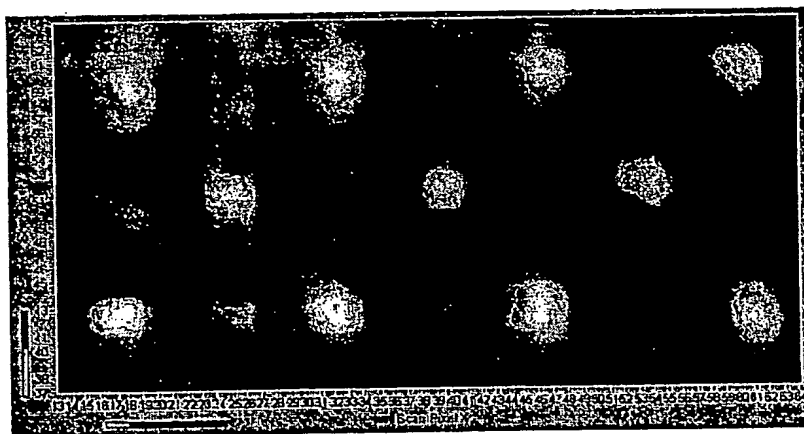


Image 5: Fluorescence intensity from position 4 showing the alternation of protected and deprotected features.

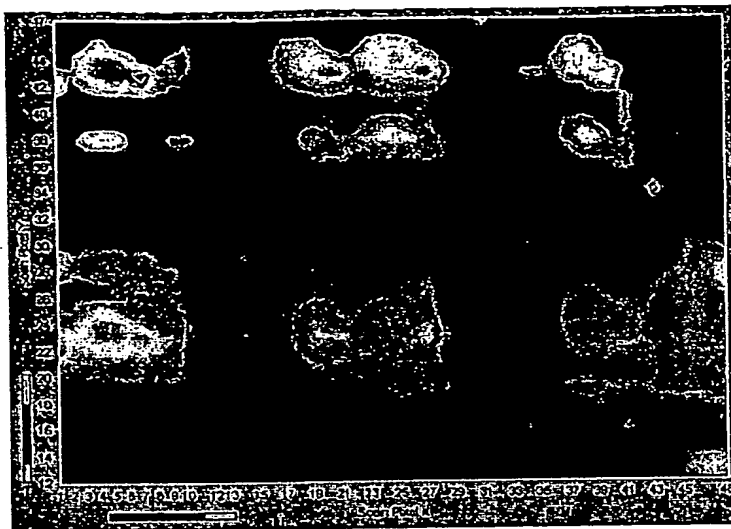


Image 6: Fluorescence intensity from position 5 showing the box like deprotection pattern.

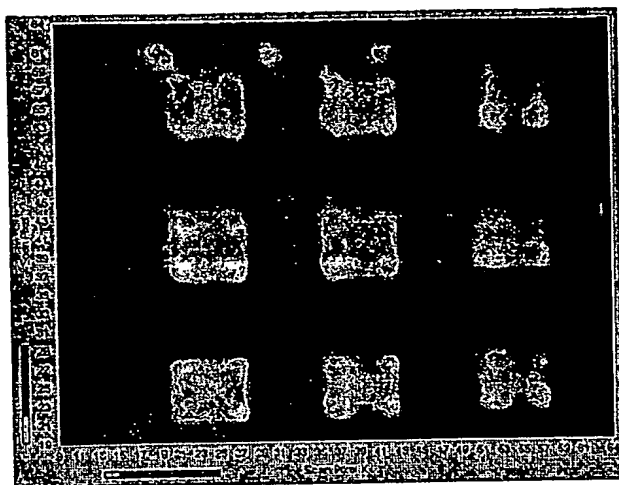


Image 7: Fluorescence intensity from position 6 showing the box like deprotection pattern.

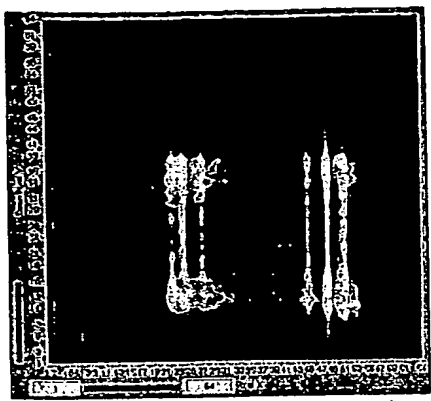
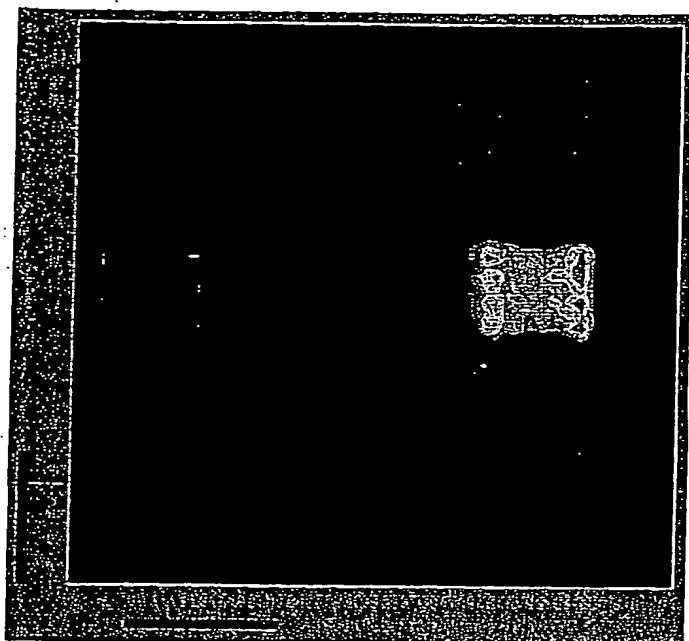


Image 8: Fluorescence intensity from position 6 showing the box like deprotection pattern of small features note that the features are 4 pixels in diameter and 25 pixel spacing, with a  $10\text{ }\mu\text{M}$  scan spacing that is  $40\text{ }\mu\text{M}$  and  $250\text{ }\mu\text{M}$  spacing as expected.



**Claims:**

1. A general method to create spatially defined complex polymer structures through sequential deprotection and addition of polymers/monomers to photopolymer structures.
2. A way of generating polymer arrays using photolabile groups with acrylate and methacrylate monomers and AIBN and its derivatives as a photoinitiator.
3. A method of enhancing the signal from microarrays by constructing the microarrays on patterned photopolymer arrays.
4. A method of generating three dimensional structures functionalized with a plurality of spatially defined functional groups.

Materials:

02/20/2004

RenShape SL 5510 SLA System from Vattico

Propylene Carbonate make 10mm or more @ 100 rpm

Isopropyl Alcohol lens w/ N<sub>2</sub>

Ti Sapphire laser 10 in FWHM 80mHz 730nm / 2

10x 0.7NA objective

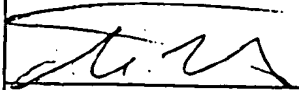
Experiment	Scan Speed	Line Spacing	Power	Size	Obs
1 'ASU'	1%	40µm	1µW	5.5 x 5.5mm	1/2
2	1, 3%, 100%	40µm	100µW	5.5 x 5.5mm	1/2
3	1, 2, 3, 4%	50µm	100µW	4 @ 1mm	1
4 'ASU'	1%	40µm	100µW	6x6mm	TIME
5	Repeated #4				
6 (Small 'ASU')	1%	9µm	10µW	500µm x 500µm	
- 16	500 x 500µm	1%	50µm	10µW	(4500µm) <sup>2</sup>
- 16	100 x 100µm	1%	10µm	10µW	(900µm) <sup>2</sup>

\* IDEAL (1) Use polyacrylamide attach peptides  
(2) or ~~epoxy~~ epoxy / methacrylate & decimate? attach peptides

make an array of PADS laser data of polymer  
with sensors

Continued on Page

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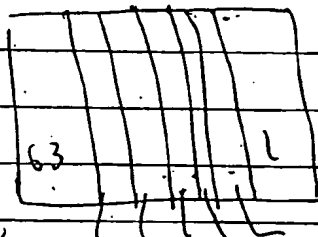
4/21/04

Date

Purpose: #1 to determine the threshold  
for photo polymerization of 2  $\mu$ m  
exposure @ 730 nm

2  $\mu$ m FWHM

Laser 730 nm output after Beam splitter before shutter = 650  
Output from objective 280 mW 0.8  $\mu$ W



'Gray Scale M'

400 nm x 400 nm 8 bit RLE

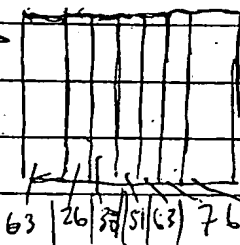
X step = 2  $\mu$ m

Y step = 6  $\mu$ m

Scan rate % 26 38 51 63 76

This worked but the pattern came off when I  
washed / wiped it. May have been focused  
above the surface.

Laser  
output  
colony  
Region  
2  $\mu$ m



Laser at 240 mW

190 nm x 650 nm

10 nm x steps

10 nm y steps

10 x objective

US22 Pattern Passes 1/13/04 as a substrate

There was some polymerization

only where there is significant polymerization

is between areas (where the sum is large, scan speeds

need to significantly slow scan speed

There was a visible border where I left more light

Continued on Page \_\_\_\_\_

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Process | See it is possible to couple a diamine to SS10  
Epoxy Resins on the SS10 polymer

213 AL 8.3 Aniso Propoxy Butane + 30 ml DMF

Exposure 6 x 9 (250 nm)<sup>2</sup> Runs w/ 200 nm Spacing 19 nm

Spacing @ 2 nm w/ 40x objective 502 SS  
Kubel of Propylene carbonate for ~ 3 nm

Exposed w/ 16A 2.8 nm w/ N<sub>2</sub>

Exposed to Diamine Solution @ 4:15 pm

Scanned same but w/ 100 nm intensity @ 100% SS

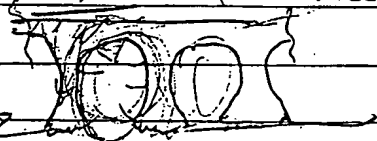
Still looks like too much

Try 10 nm for 1st 3° (over)

Switched to 1° for the next

Threshold

@ 10 nm  
made Archival  
like this



didn't see anything @ 1.4 nm

need to either start with polymer or use two photos  
overnight

- Sample left in diamine & then dissolved off glass  
residue a small piece & washed in methanol  
& placed in light - very soft with curved

- may want to cure while reacting w/ diamine

6:50 am - Added two samples (1 w/ & 1 w/o diamine) to 100% w/ diamine  
8:00 am - added 6 x 9 w/ DMF

Exposed 50 w/ methanol

+ diamine is unbelievably expensive - not very if at all fluorescent

so this may be a possibility

Epoxy has good resistance to  
solvent solutions (Hexane,  
DCM, THF, Acetone, etc.)

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Not DMF

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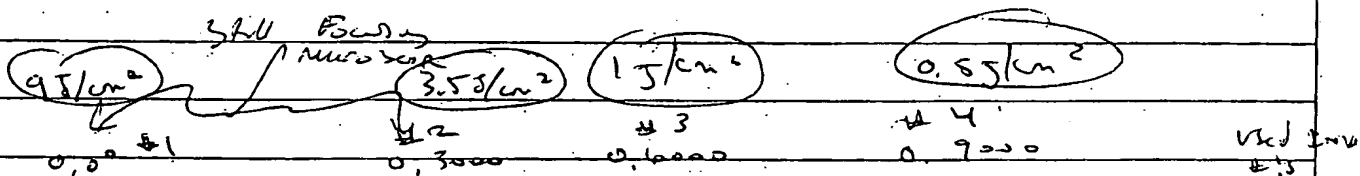
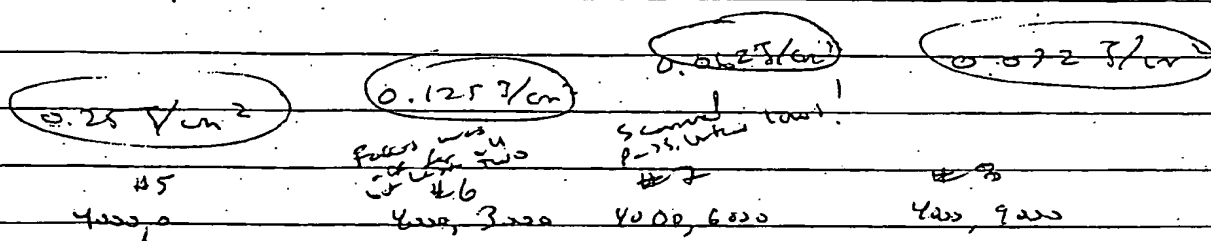
Date

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Date

SWL in = SWL out of objective in  
SWL in = 1 mW at 400

Start w/ SWL input ~ 1 mW output from 400. 1 J/cm<sup>2</sup> @ 88% SR  
Start high & go low. Curves measured w/ black 60W  
1st SWL 21



Scan	Size	SWL Input	SR	SWL Output
1 <sup>st</sup>	300 x 300 w/ 150 spacing	1 mW	88%	0.88 J/cm <sup>2</sup>
2 <sup>nd</sup>	Same	0.5 mW	88%	0.44 J/cm <sup>2</sup>
3 <sup>rd</sup>	Same	0.25 mW	88%	0.22 J/cm <sup>2</sup>
4 <sup>th</sup>	Same	0.125 mW	88%	0.11 J/cm <sup>2</sup>
5	300 x 300	2.5 mW	90%	2.25 J/cm <sup>2</sup>
6	Same	1.25 mW	90%	1.125 J/cm <sup>2</sup>
7	Same	0.625 mW	90%	0.5625 J/cm <sup>2</sup>
8	200 x 200 50 lines SWL strong	3.13 mW	90%	2.81 J/cm <sup>2</sup>
9	Same	1.56 mW	90%	1.405 J/cm <sup>2</sup>

\* More of Sigmant Scatter off top can stay!

add edge to my/mi input + seal area but not for 20 mW

\* Focusing is very difficult  
went up ~ 100-180 mW  
By 150 on the size  
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Craig Chaper 4/21/04  
Signed \_\_\_\_\_ Date

Continued on Page \_\_\_\_\_

Insburg v1 545 RF 35 w/ 50000 Power input  
Dist is 700 Pluviusent inches (200-1600)<sup>2</sup> 15x1.5  
LTS of Precipitation (probably 2000-4000) on Surface  
Inches w/ 120000

Dist from	Insburg intensity	Background intensity	Comments
1 (Inches) $x=12$	100-250	100-250	No visible pattern
2	381	134	
3	253	134	
4	128	129	
5	155	129	
6	155	129	
7	155	119	
8	155	119	
9 (90000)	155	119	

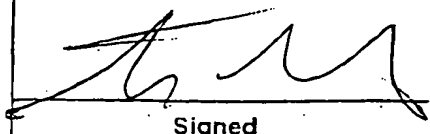
Overall observations: Very Spatchy

I don't think this insubtle program is really  
work #9 was very flat <sup>on the map</sup> yet on the Bilt Corp  
it looks like it has the same topography as the other

Mount Baldy is working well #1 looks the  
best how to make out any features after #3  
27 J/m<sup>2</sup> might be perfect

Continued on Page

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4/16/04

Date



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4/21/04

Date

try using more concentrated diamine in TAC. For a short  
 test - 426  $\mu$ L Bis-Amine properly before in 100% TAC  
 ~180 mM

first scanning at <del>1.0 mW</del> w/ 10x (2000 $\mu$ m) <sup>2</sup> 25x25	✓
1 $\mu$ S exposure	
Scanning 0.5 mW	same ✓
0.25 mW	same ✓
0.1 mW	same no

the strains were too high! they fell atop each other

Trail of 40x (2000  $\mu$ m)<sup>2</sup> 20x20 lines

they don't stick to the glass very well  
 may also be APR8 surface

keep working w/ 10x ... can always (hopefully) scale down  
 250 mW 10x 1 ms/pixel (4000  $\mu$ m)<sup>2</sup> 20x20 pixels  
 200 nm between spots

put on PC Agitated gently until excess had dissolved  
 put microscope lamp on it for 5 mins while in the  
 PC

Automatic Scans to Ammonia 5510 very well  
 125 mW w/ 90% SR! 10x is light at the threshold  
 only the edges polymerized.

gives us more intensity

	0.2
	0.3
	0.5 4000 / 200
	0.75
	1 mW

Trail making some little "Hem"

Continued on Page

1st scan @ 0.2 mW Read and Understood By

2nd scan @ 0.3 mW

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Date

4/21/04

Worked with  
 through  
 Signed

800 mW  
 10x  
 75  
 4/21/04

1000  $\mu$ m x 4000  $\mu$ m 25x25 lines

PROJECT 3/15/04

Notebook No. \_\_\_\_\_ 73  
Continued From Page 71

Measure 'Haze' from pg 71

w/ DAPI filters exactly @ 365nm Get a 'Huge' fluorescent signal. Probably from photo initiators.

need to take an excitation / emission spectrum from polymer to figure out if I can find a dye to use with it / where it will have a small background. Also see that it is generating white light.

Measure w/ 40x 40mm lenses all 25mm in diam. Have a S/N of 80 Much better 1160 = 5 N = 38 <sup>Dark Count = 3</sup>

What would happen if you spinned beads into this? You would have a SPR system?

$$\frac{1160}{13} = 9$$

Power dependency of emission

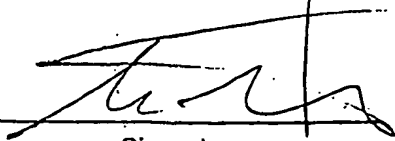
Input Power (mW)	APC	Input Power (mW)	APC
0	7.5 E 2	90	7.6 E 6
2	7.8 E 2	80	2.5 E 6
3.4	8.7 E 2	70	2.3 E 6
5.4	9.8 E 2	60	2.2 E 6
8.6	1.1 E 3	50	2 E 6
11.2	1.2 E 3	40	1.8 E 6
16.6	1.4 E 3	30	1.5 E 6
23.4	1.7 E 3	20	1.1 E 6
39	2.3 E 3	10	6.4 E 5
		7.7	5.7 E 5
		6.3	4.8 E 5
		3.6	2.9 E 5
		1.2	1.1 E 5
		.8	7.2 E 4

Not well captured

Continued on Page

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1.5 E 3



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4/16/04

Date



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4/21/04

Date

3/16/04

[illegible][illegible]

## PROJECT

Notebook No.

Extraction

Extraction @ 550

Extraction

Continued From Page

Extraction @ 500

450	2423	450	3341	
475	2966	475	5039	
500	3567	430	2470	
425	2100	420	3055	
400	1781	410	5234	
365	1083	400	3437	580
350	515	365	2771	420
		350	1282	460

100 mm 8.165 8.167

Extraction

Extraction

8

350

450

1295

400

478

2913

425

500

2278

450

527

2177

475

540

1492

500

534

2330

365

447

2726

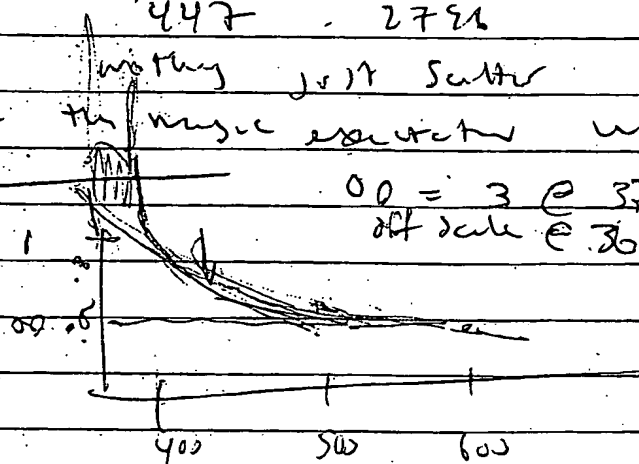
550

nothing just scatter

(515) may be the magic extraction number. Need to see  
low level

00 = 3 @ 375 mm  
off scale @ 365 mm

Agriculture



Continued on Page

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Date

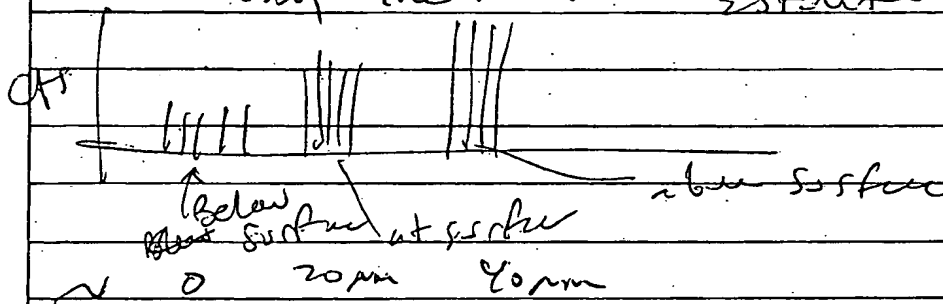
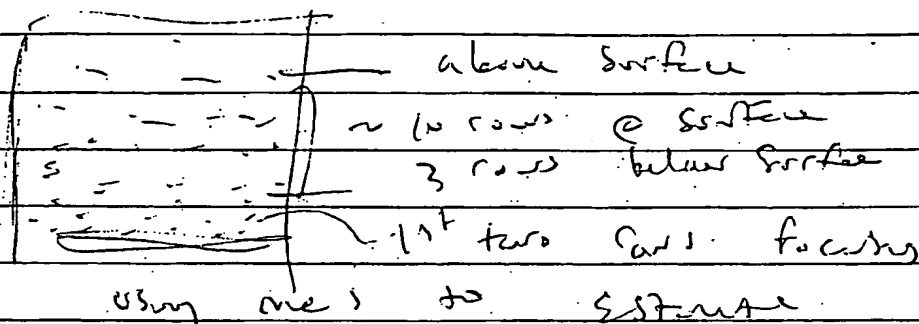
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4/21/04

Date

13mW input w/ 40x objective 36.5m excited

APTES Slur

3000 x 3000  $\mu\text{m}$  30 pixels x 30 pixels Shutter open 1 ms

Proceed into bed of automatic

need for to see how put over a light (or microscope)  
for 5mFibers are 5  $\mu\text{m}$  wide & 2  $\mu\text{m}$  long.

And to make longer fibers by using thicker  
 coating placed 2 150nm (and 515 on APTES slide  
 & used a 3" x 2 CS to spread ~40% of polymer on  
 surface

30mW power  $(1000 \times 1000 \mu\text{m})^2$  25-28 m.s. 10ms / pixel40  $\mu\text{m}$  spacing

Worked well except random fiber over ~80nm long 1.25  $\mu\text{m}$   
 Continued on Page 76

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PROJECT Attempts to make features flat

start - up By coloring in place stay low intensity for a long time.

137/mw for 2hr w/ Snc-500

From above 71 rather than high intensity

300mw for 1ms try (10mw for 30ms)

if this doesn't work try

Rising force; once to pattern of the beam from to cure

□ 3ms

□ 300ms

Failed using mes lens. □ 30ms

10mw 10x objective  $(1000\mu m)^2 / (10\mu m)^2$

30ms @ 300mw, 3ms

3 (only one that worked, thought it still works but starts up.

Cuts

Features were ~ 25μm in diameter

insulated w/ 100mw for 1ms w/ 40x

worked well!

2 sec @ 100mw  $(1000\mu m)^2 / 10^2$  } Both appeared to  
3ms @ 100mw 3x  $(800\mu m)^2 / 8^2$  } work  
Don't start up as well

insulated w/ 40x: 40mw too much power

Very large fibers ~ 2200μm long 20mw w/x

insulate 400x400μm / 30x30 @ 10mw w/ 40x

~ 20μm across ~ 200μm long

Continued on Page

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This is how to how done the beam is 13x 4/16/04

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probably 10mw x 150μm is how a couple.

Now try w/ 40x

VB 8/cm

$$\frac{\pi}{4} \frac{(10 \times 10^{-6} \text{ W})}{(12 \times 10^{-4} \text{ cm})^2} = \frac{33.8}{26.5} \text{ J/cm}^2$$

mess 800ms w/ 2.1W input w/ 40x  
 send so w/ 8.2W input

The nice method of following works well: looking for  
 the maxima closest to the surface  
 everything has collapsed!

244ms 82ms to work well for various Polym

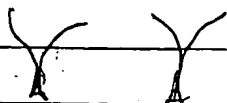
30W 40x	1ms	0	0	
" "	1ms	10ms	1000	500 x 500 nm / 10x10 pins
	1ms	100ms	2000	0
	10ms	10ms	3000	0
	100ms	100ms	8000	8000
	100ms	0	2000	2000
	200ms	0	3000	3000

send the 5510 by blowing Argon across the surface.

quenching the

only the 100:100 bracket?

Still few are interestingly our pattern come out  
 as turning,



Continued on Page \_\_\_\_\_

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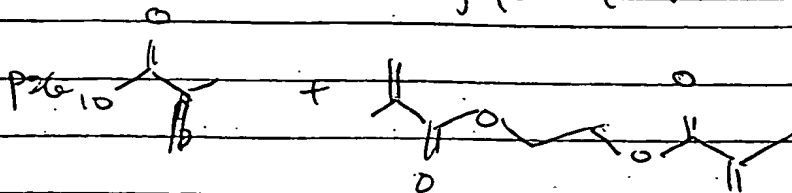
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P2610 MA

EGDM

1 <sup>st</sup> attempt	2 <sup>nd</sup> attempt	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
P2610 MA	0.9 ml	450 ml	45 ml	
EGDM	19 ml	10 ml	10 ml	100 ml
AZOIBN	11 mg	0	2	10 mg
Ethanol	1 ml	0	6	5 ml
Benzophenone	0	6 mg	28 mg	5 mg
	Nothing	Nothing	Some film	worked
			formation?	after a while

NEED TO THINK ABOUT KINETICS... NEED  
TO REMOVE INHIBITOR!

NEED ANNEAL Triethylamine @ 50 mM

1% initiator

5<sup>th</sup>2<sup>nd</sup>

50% monomer

	units	1	2	3	4	5	6	7	8
EGMA	50-98%	98	98	98	98				
AZOIBN	1%	1	0	0	0				
PB	1%	0	1	1	1				
TBA	0.5-1%	0	0	1	0				
BAPPB	0.5-1%	0	0	0	1				
ETOH	0-48%	0	0	0	0				

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monomer, initiator, and anion

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demanded inhibitor by Canny over Sigma-Aldrich  
failed.

first washed column w/ 2 to 4  
for before: discarded 1st part  
flushed w/  $N_2$   
kept under  $N_2$   
polymerizing in Beaker

$$\left( \frac{50 \text{ mmol BP}}{L} \right) \left( 182.2 \text{ mg} \left( 0.001 L \right) - 9.11 \text{ mg} \right) \sim 1\%$$

① 10 mg Azobisisobutyronitrile + 100  $\mu$ l Cytochrome c + DMF + 900  $\mu$ l MA

② 10 mg BP + 10  $\mu$ l TGA + 1  $\mu$ l MAG

200  $\mu$ l ① + (10 mg Polyethyl pyrrolidone + 100  $\mu$ l DMF)  
200  $\mu$ l ② + " "

The samples w/ PVP worked significantly better than  
those without.

made  $\frac{200 \text{ mg}}{200 \mu\text{l}}$  Azobisisobutyronitrile in DMF  $\frac{200 \text{ mg}}{200 \mu\text{l}}$

made  $\left( \frac{10 \text{ mg PVP}}{200 \mu\text{l} \text{ MMA}} \right)$


added 50  $\mu$ l of Azobisisobutyronitrile solution

worked ok, not sure if it is any better than 1%  
need to check even more.

need controls to understand what is happening

Continued on Page \_\_\_\_\_

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	MAA	DMP	PSP	BL	A20	TEA
1		X	X			
2		X	X		X	
3	X					
4	X				X	
5	X		X		X	
6	X	X		X		

#5 long AP + 10 ml of 20

A20 of DMF + 180 ml MMA

Exposed 2nd input for 3 min w/ 10x

OBSERVATIONS:

Before Wash off Excess #4 & #5  
 Have clearly material on top. The others  
 are clear

4, 5, 6 are the only ones that polymerized  
 4 is white!  
 5 is clear large before very nice  
 6 is very small & clear.

Formulation Number 5 seems to be a good  
 starting point.

Exposure test with Formulation #5 2.1 min w/ 10x

1 sec Barely visible spec

10 sec Hair &lt; 100 nm wide probable close to 20

30 sec ~ 200 nm

70 sec 1 mm wide

Keep watch for new

Continued on Page \_\_\_\_\_

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Try adding Diamine 1%

① 10mg PVP + 2mg PZDA (1500g/mol) + 10ml DiAzo in DMF + 178  $\mu$ L MMA

② " 2  $\mu$ L Bisoxazone Propoxy Butene " "

PATTERNED ①: ②: #5 from before 2.1 mW 10x 30 sec  
washed w/ acetone

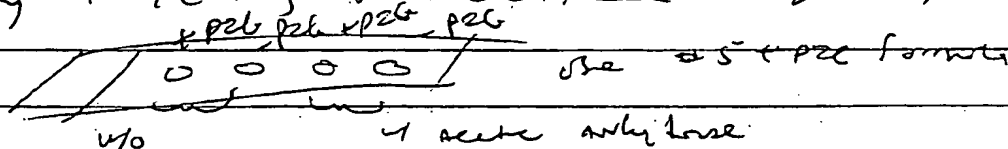
all spots look the same

Stained w/ 10mg DAPI in CH + 500ml DMF + 10ml PZDA  
430nm left 1 of two #① washed one on edge  
445nm

See figure, but Both TATZ #5 + PEG + DAPI 1 g  
#5 + DAPI are fluorescent #5 + PEG is  
1/10 as fluorescent (excitation @ 365nm, 300nm W Yaw)

This maybe ok. Need to determine if the dye  
is attached w/ surface or is simply being adsorbed.

TEST By Acetylating the surface & staining w/ dye



Exposed to 2.2 mW 10x for 3 sec

+ PEG Hx as full ad - PEG

Alcetic Anhydride Soln: 0.9ml DMF + 100ml AA + 100  $\mu$ L PZDA

Dissolve polymer (cured) w/ 1 5 marks

cured polymer - allowed to dry overnight under desiccator

& it swells in DMF - goes to remove dye.

Needs to cure under lamp for ~ 30 min Continued on Page  
To conclude.

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Load GMA on Column 18" x 0.5"

1st run 10 ml on F

2nd 10 ml 200

3rd 10 ml 200

For GMA, did not polymerize w/ 1% Azo

Run 4 Success Time (re run)

	% w/ me				Result
	A20	mm	GMA	PEB DMA	
1	X	X			works turned white?
2	X			X	Clear w/ 30 sec
3	X	20 60		40	worked well fast!
4	X	80		20	
5	X		80	40	Slow (60 sec) Clear
6	X		X		Nothing
7	X	20	5	75	similar to 9
8	X	30	20	30	very slow (slowest)
9	X	35	5	60	turned the best. slow
10	X	75	5	20	turned white @ 30 sec

Added 10% BAPS in run to #7 w/ 10 ml had  
formed like Gel prod. i.e. most was still un-reacted?  
probably dissolved in DMP & reacted w/ DMP.

Core over temp for 15 min + repeat ✓

re-run #9 - 10 min for 5 min, no apparent change  
in physical properties

Molded w/ DMP #1 (#8 &amp; #9) for 15 min

Continued on Page \_\_\_\_\_

Cured w/ Acetone: thin

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2X w/ DMP

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Very thick &amp; #8 is not.

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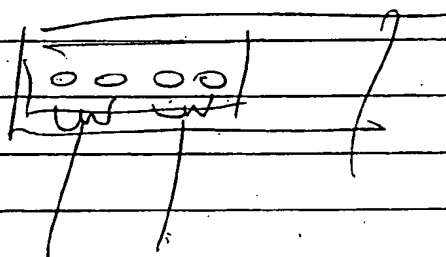
This is good & 9 looks like a good finish, may want to  
 let GMA to triple check that the inhibitor is removed.  
 There needs a way to attach to the alkyl. & need  
 a way to confirm.

Make more of #9 using GMA that has been  
 stored w/ Aldrich beads for 1 hr

Don't like how the resin turns white  
 try w/ MMA

Azo	GMA	poly DA
100%	100%	850%
X	50%	850%
100%	50%	850%

OOPS used up adding 2% Azo



1 2 20% of each.

Has been fluorescent not after  
 P26-NMA has become fluorescent! probably from B2H  
 60 sec of wts. stand in. everything mixed together  
 exp messy pattern

Added 10% BAPB 1253 pm

Try 50% Azo + 100% GMA + 850% MMA

Continued on Page

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→ Poured more PEGDMA over Sigma tested  
 → Recrystallized Acrylic isobutyrate from MeOH @ RT (AIBN)

Recipe ① Some AIBN + 200  $\mu$ m PEGDMA + some GMA

② " " + some GMA + 900  $\mu$ m PEGDMA + 700  $\mu$ m MAA

Some AIBN + 200  $\mu$ m PEGDMA + some GMA + some BAPB

→ Cured for 60 sec @ 2.5 mW

→ Measured both w/ DSC. Both were stored @ 2 mm from #1

→ This makes a soft polymer, probably the BAPB is the cause the material -

→ The MAA seems to make it cure faster & more rigid

→ May be that I'm using too much light & getting short polymers.

Try less light & blinding w/ some MAA

Use 200  $\mu$ m for 60 sec w/  
 1% AIBN + 1% (BAPB + GMA) + X

X = 10 - 100% MAA ; 0 - 70% GMA

Continued on Page \_\_\_\_\_

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Real work of ~~the~~ GMA through Aloride resin  
disperse column (0.5" x 8")

Repacked w/ from resin for PGE DMA

mass 1.2 Arbut (a crystalline from most) solvents.

Bubbled  $N_2$  through for 2 min

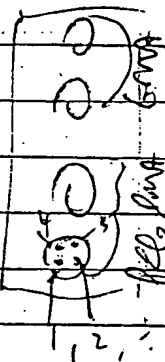
placed on cold slide w/ cold partition in sand  
w/  $N_2$  blowing through over it at  $85^\circ C$

11:45 am 4/6/04

12:45 pm Both samples cured into clear hard polymers  
The PGE doesn't stick to the glass very  
well can't cut the GMA off the glass  
All three are like little insulating  
leaves

1:45 pm @ 365 nm 2 min input into processor

Went sample to 30% over to me I put it in  $N_2$  for  
twenty minutes later found to 500-1000 nm



1 = 30 sec @ 2 mW

2 = 30 sec @ 200 mW

3 = 300 ms @ 200 mW

4 = 4 @ 2 mW

Continued on Page \_\_\_\_\_

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4/20/04

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None of the light induced polymerizations today worked very well. The GMA sticks to the Glass like crazy. The PGE doesn't appear to swell very much in DMF or water.

together they might work. Try various concentrations in DMF

	PGE (wt)	GMA	
1st	100	0	None. Don't stick to glass
2nd	0	100	Stick to glass amazingly well
①	50	50	then Sparged w/ $N_2$ for 1 hour Then spatted on plate in DMF @ 280 Pours w/ $N_2$ Finished in 15 min.
②	99	1	
③	90	10	
④	75	25	

None stuck

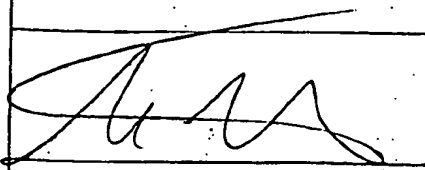
All are clear. At 1<sup>st</sup> set in BAPS (melt) 620 in 4/6/04

only Takes 10 min but need to sparge  
The 90/10 cracked overnight, may not have anything to do w/ bubbles

The set treated w/ Diamine is very fluorescent  
even after washing 2x w/ Acetone - 300 w/ DMF  
The set not treated w/ Diamine is not fluorescent

Continued on Page

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4/26/04

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w/ 120 AIRN

	<u>IMA</u>	<u>PEOMA</u>	<u>GMA</u>	
A	100	0	0	TMA 378.4 mg/m <sup>2</sup>
B	90	0	10	PEOMA 198 mg/m <sup>2</sup>
C	45	45	10	GMA 142 mg/m <sup>2</sup>
D	49	0	1 3.5	

A & D are very brittle, they crumble

B & C are much better

B + 10% Baux in AME 220 pm

E 75

f  
Regressed 4 zone spots of B in area (left) for 30 min  
@ 350w like for 5 min (350w) The plate  
as slide 4 zone spots for 5 min  
put in plate dish on ~~plate~~ dish w/ plastic  
curved w/ thick cloth

~~30 min degressed~~  
~~30 min degressed~~

300w 50w	0
300w 50w	50
300w 50w	0
300w 50w	0

0	1 sec @ 300w output <u>worked</u>
0	1 sec @ 300w no output work
0	300w 30 sec <u>worked</u>
0	75% SS @ 300w (300w <sup>2</sup> ) 12 not

300w did not work  
300w 50w

appears that you need a lot of light! Continued on Page

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Signed Date Signed Date

Took a methacrylate slide put some of the polymer on it  
Scanned area of  $(1\text{mm})^2$  1000 lines @ 3 mW  
all @ 3.2 mW

	$(1\text{mm})^2 / (100)^2$	99.2	SS	@ Focus
	$(1\text{mm})^2 / (50)^2$	99.2	SS	
		50.6	SS	
		25.2	SS	

Made more B 900 ml TMA + 100 ml GMA + 10 mg AIBN  
saturated w/  $\text{M}_2$  for 5 min then  $\text{H}_2$  through overnight  
placed slide in oven when  
polymerized

04/13/04	TMA	2 mm ethyl methacrylate	AIBN
my MTA soluble	50	50	12
	90	10	22
	95	5	
	99	1	

04/14/04 Scanned w/ 10x 370 nm  
3 mW 99.2 Sc Rate

(500 nm)/10 w/ 600 nm spaces  
w/ 100 nm

etched/developed w/ other worked ok

Continued on Page

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A hand-drawn diagram of a circular structure, possibly a cell or a microorganism. It features a central rectangular area with internal components labeled with letters and numbers. The diagram is drawn with simple lines and includes a scale bar at the bottom right.

2 250/25 *semi*

3 50/100 Same

4 500 / 2500      worked Best      same  
500nm spacing

Worked w/ ether

only see separation of features in # 4

• 10% BAPB in  $\text{CH}_2\text{Cl}_2$  for 15 min

As it's not a loss. Share b have

Conc 1st (wound to 50% Bling w/ Thresh)

Handl Brk 3x

19 May Moz & local pipit + small birds


5:50 am - 5:40 pm

Cont 30 Dmf

Filed at De Bore

Deflected 2 signs on path # 7

۱۲۷

5 mW  $\rightarrow$   1 mW by 5R 10 lines

□ □ □

10 mg Dantrol + 10  $\mu$  M PZA + 500  $\mu$  M DNF 18 ml  
 total 20x by DNF to get dye off (Continued on Page 2)

Continued on Page

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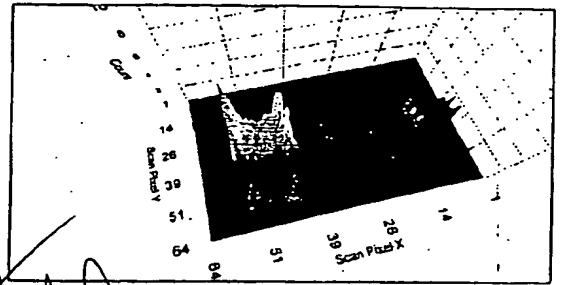
Date \_\_\_\_\_

PROJECT \_\_\_\_\_

Notes Book No. W/ 100 mW  
Continued From Page  
370am 6x no include

93

The two positions reported  
are more fluorescent than  
other positions. Though swimming  
fishes are more fluorescent  
(thick scatter) & none are  
non fluorescent (w/ complete  
protection).



There is a difference between  
the 3mW & 1mW.

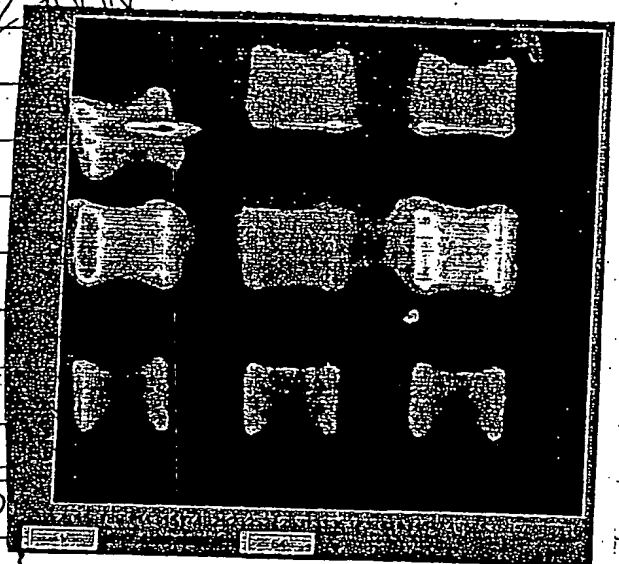
no more species? see

3mW

500/650 w/ 750am species?

AA Cap after more protection?

why don't all of the  
features adhere to the  
glass?



marked 4-15-04 after  
overnight in DMF 4mW 100  
no pin hole

Using 400 I can see that there are differences in  
thickness as a result of the scan spacing. They  
appear to be ~10mm long w/ lower species.  
it is further interesting to note that features  
1 & 2 are fluorescent as if the dye or protective  
glass was/were not able to protect (Continued on Page 97)

(also was Read and Understood By [Signature] 1 was)  
4/16/04 Anne  
Signed Craig Maguire 4/21/04  
Signed \_\_\_\_\_ Date \_\_\_\_\_

This addresses:

- ① repeat but cap w/ AA
- ② make more hydrophobic polymer (increase from PEG w/ PEG)

Try both

TMA / PEG / GMA ~~45/45/10~~  
 950m / 450mL / 45mL 11mg AIBN

~~DESS~~ 15mL (BVB66W / N<sub>2</sub>)

used mes

10 PMS 5mL

- ① pour 500 / 25 w/ 1mm spheres @ 100 mW 50% SR
- ② wash excess w/ ether
- ③ blow N<sub>2</sub> through! Heat to 50°C for 15 m
- ④ mabz
- ⑤ 10% BAPS w/ DMF 30m
- ⑥ N<sub>2</sub>O 30m
- ⑦ AA 15m
- ⑧ repeat some other factors w/ 3m
- ⑨ Purulite 15m
- ⑩ rise many times w/ DMF

1,4-Bis(3-oxo-2-propenyl)benzene

Continued on Page \_\_\_\_\_

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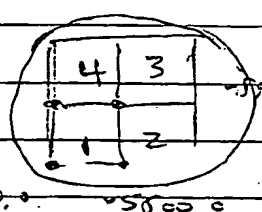
Signed

Date

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Date

- ① The med is getting 60 counts ~ 6 hrs what it was before  
retention of the is from previous pre-hole or that the  
pelt is fluorescent.

	①	500/25	w/ 100mm spray	792 SK
	②	"	"	662 SK
	③	"	"	332 SK
	④	"	"	17 SK

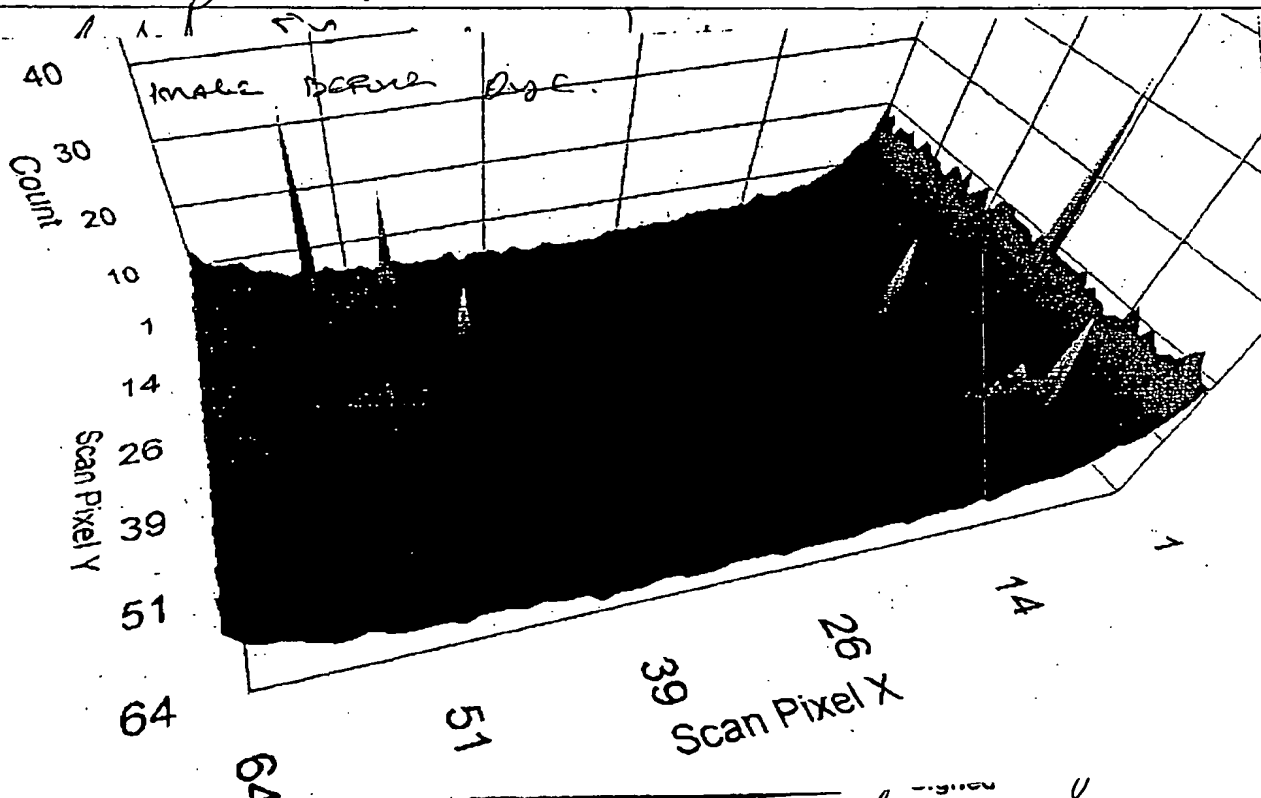
#3 is more fluorescent (2x) than ① & ②. Why? because of an  
exposed surface

- ② - was 5x fluorescent before! for the w/ p.p.H  
w/ EtA2

bleach w/  $N_2$

seal chamber in vacuum oven

- ③ we moved to 55°C for 15 min while blowing  
 $N_2$  through oven



30mm m  
table @ 200x  
0 370 mm

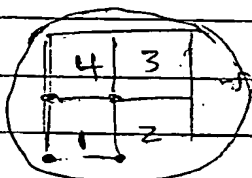
Page

x/04

Date

PROJECT \_\_\_\_\_

① The mes of getting 60 counts ~ 6 times what it was before  
notion of Res is from beams pre-hole or that the  
pel is fluorescent.



①	500/25	w/ 100mm spray	792 SR
②	"	"	662 SR
③	"	"	332 SR
④	"	"	12 SR

#3 is more fluorescent (2x) than ① & ② which are in  
beam source

② 100mm 5x fluxing back: further w/ p.p.H  
w/ EtA2

blew dry w/ N<sub>2</sub>

seal chamber in oven over

③ warmed to 55°C for 15 min while blowing  
N<sub>2</sub> through oven

boxed 5x spray on - out

④ ~~boxed~~ ⑤ 4 ml MDC + 10 ml AP24 + 50 ml surf 30 min  
in table @ 200 rpm

10x objective 992 SR 100mm input power @ 370 mW  
features are ~ 9 counts

⑥ CAP: 146<sup>30</sup> ml AP24 + 20 ml AA + 1 ml surf 15 min  
on orbital table @ 200 rpm

Continued on Page \_\_\_\_\_

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⑦



Defect # 4

50%

252



  

10 lines for definition

LT  352 

up 10 x @ 2 mm. net

12.10mm  280  280

992

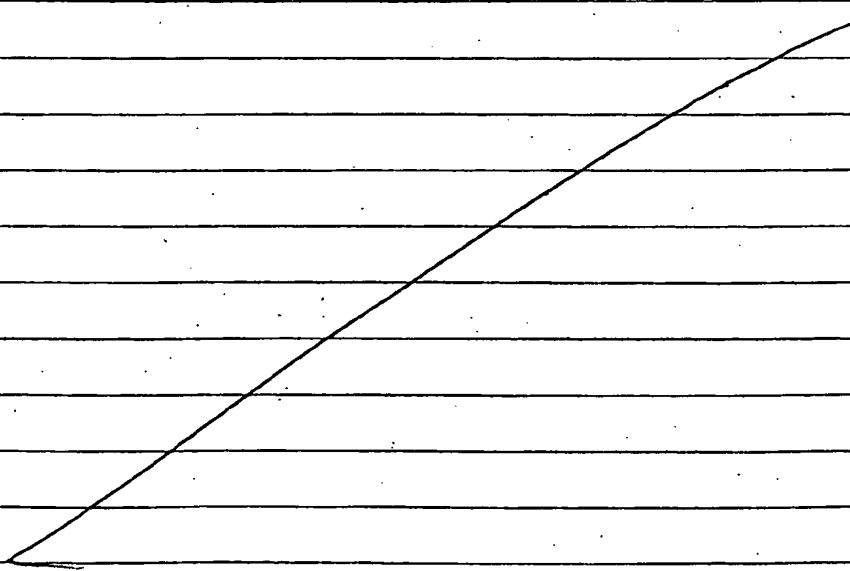
1 1/2

Panel 320 up and

⑧

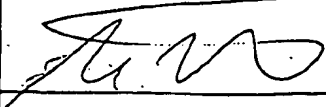
10 day survey + Sound after + Sound and  
15 min. on table @ 200 RPM

Consistent test for off at bottom. A larger  
by right under better later w/ this system



Continued on Page \_\_\_\_\_

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4/21/04

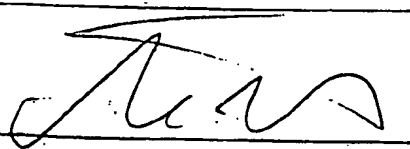
Date

base to pattern pyc on photo polymer.

- I have been able to make small polymer features w/ the 5510 polymer, as small as 2  $\mu\text{m}$ . I have also made small features w/ my own methacrylate/AIBN formulations but I haven't quantified their sizes yet.
- I have imaged long 'hairs' of 5510 photo polymer w/ aspect ratios  $\sim 100$  with a Confocal microscope & they appear to act as optical fiber like I see a huge fluorescence when imaging at the glass polymer interface.
- I have successfully protected and deprotected my methacrylate resin functionalized w/ A diamine. The protection was w/ NMO & deprotection w/ the same laser? Utilization using optical fiber. This should provide significant improvements in my contrast ratio vs. working on functionalized glass. Further I hope the surface chemistry will be better vs. glass so I will be able to synthesize long pectrs. w/ high yield using the NMO protected-deprotected scheme.

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4/16/04

Date

Craig L. Magee

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4/26/04

Date

purpose: to screen formulations against the following criteria

- ① rate of release of Absorbed dye (case of wash)
- ② yield of MOC protected acetone

Methodology: Prepare samples, set onto Rm 8 4 well plates, ~~Ammonia~~ but to  $50^{\circ}\text{C}$  for 15 min with blowing  $\text{N}_2$  through over. React w/ Diamine, MOC, & ready 1 or 2 or 3 or 4 or 5. React w/ Solvent (DMF) & look for maximum fluorescence. Test Best formulation for Cefixime Against Diamine

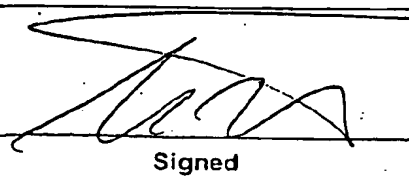
Samples: All 12 ABN Samples. make STD solution of 12 mM GMA

Trinitrobenzoyl chloride (TMA)      Glycidyl methacrylate (GMA)

#		
1	10	90
2	20	80
3	30	70
4	40	60
5	50	50
6	60	40
7	70	30
8	80	20
9	90	10
10	95	5
11	100	0

Continued on Page 99

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4/20/04

Date

Craig C. Mayall

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4/21/04

Date

Date \_\_\_\_\_

[illegible]

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APPLICATION NUMBER: 60/608,774

FILING DATE: *September 10, 2004*

RELATED PCT APPLICATION NUMBER: PCT/US05/15764



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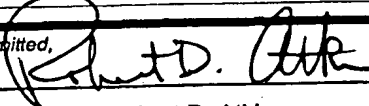
**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**  
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).17497 U.S. PTO  
60/608774

091004

INVENTOR(S)					
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)			
Trent Russell	Northen	Tempe, Arizona			
Additional inventors are being named on the ____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
LIGHT ACTIVATED MOVING POLYMER					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number 26707					
OR Type Customer Number here					
<input type="checkbox"/> Firm or Individual Name					
Address					
Address					
City		State	ZIP		
Country		Telephone	Fax		
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages 12		<input type="checkbox"/> CD(s), Number			
<input type="checkbox"/> Drawing(s) Number of Sheets		<input type="checkbox"/> Other (specify)		Cover sheet; Postcard	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE AMOUNT (\$)	
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055				\$80	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

Respectfully submitted,

SIGNATURE



TYPED or PRINTED NAME Robert D. Atkins

TELEPHONE (602) 229-5311

Date 09/10/04

REGISTRATION NO.  
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Docket Number:

34,288

112624.00147

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Applicant: Trent Russell Northen

Date of Filing: September 10, 2004

Title: *LIGHT ACTIVATED MOVING  
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**PATENT**

**PROVISIONAL APPLICATION**

**Of**

**TRENT RUSSELL NORTHEN**

**For**

**UNITED STATES LETTERS PATENT**

**on**

**LIGHT ACTIVATED MOVING POLYMER**

**Attorneys:**

**QUARLES & BRADY STREICH LANG L.L.P.**

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## Light Directed Movement of Polymer Microstructures

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### Abstract

Light induced surface chemistry changes have been used move swollen polymer microstructures. Swellable trimethylolpropane trimethacrylate (TRIM) crosslinked poly(2-hydroxyethyl methacrylate) conical microstructures were constructed by azo-bis-isobutyronitrile (AIBN) photopolymerization using a 20x 0.5NA microscope objective and 365nm laser excitation. Structures were aminated with glycine and protected with the photolabile group 4-nitroveratryloxycarbonyl (NVOC). Differential swelling with and without NVOC of 10% was observed in N,N'-dimethylformamide (DMF). Removal of NVOC with 365nm laser excitation induced polymer shrinkage in excess of 4%, resulting in maximum polymer velocity of 1mm/s, and displaced solvent velocities in excess of 0.01mm/s.

### Introduction

A number of synthetic polymers have recently been developed that respond to changes in surface energy resulting from external stimuli including: mechanical deformation, heating, solvent contact, and exposure to light[1]. Exciting applications of such materials include: implants based on shape-memory materials, gels respond (e.g. swell) in response to changes in pH or specific molecules may be used for feedback control for drug delivery, and microfabricated vascular networks[2]. Given the fact that three-dimensional polymer structures can now be constructed on the submicron scale using nonlinear laser patterning [3, 4] [5, 6], it should also be possible to develop micro or nanomechanical devices based on polymer movement.

A particularly versatile stimulus that could be used for directing polymer movement at dimensions down to the submicron level is light. Photolabile protective groups offer the ability to selectively break bonds using light and therefore substantially change the surface characteristics of the polymer in a light-directed fashion. 4-nitroveratryloxycarbonyl (NVOC) is a common photolabile group and is known to cleave using a Norrish-type II reaction [7]. It has found wide use in protecting amines [8] and has applications including: photogeneration of organic bases [9], microarrays [10], novel proteins [11], and variations of NVOC as linkers in peptide synthesis [12]. In these cases the addition and removal of NVOC modulates the reactivity of an amine. This work describes the use of NVOC to instantaneously modulate the surface properties of a porous polymer using light.

Porous polymers are common in solid phase synthesis [13], drug delivery [14, 15], tissue engineering [16], and separations [17] [18]. A range of polymers are now used for solid phase synthesis including polyacrylate resins [19]. These porous polymer structures are the result of phase separation during free radical crosslinking copolymerization swell in

### Light Directed Movement of Polymer Microstructures

compatible solvents [20]. Typically solvents (porogens) can be used to control the pore size [21]. The surface of the polymer is often modified to improve functionality [22] or as a result of solid phase synthesis.

Here we report polymer microstructures that shrink and move when illuminated with light. Cleavage of NVOC immediately exposes the primary amine, resulting in large changes in the surface chemistry and swelling of the polymer. This allows light-directed spatial control of polymer movement.

## Experimental Details

**Materials:** Glass coverslips for an FCSII chamber (see below) were purchased from Biopetechs (Butler, PA). 2-hydroxyethyl methacrylate (HEMA), trimethylolpropane trimethacrylate (TRIM), azo-bis-isobutyronitrile (AIBN), piperidine, diisopropylethylamine were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI). 4-nitroveratryloxycarbonyl chloride and 3-(trimethoxysilyl)propyl methacrylate were from Fluka GmbH (Buchs, Switzerland). Dimethylformamide (DMF) was from Applied Biosystems Inc. (Foster City, CA). Methanol, hydrogen peroxide (30%), sulfuric acid, hydrochloric acid were purchased from Mallinckrodt Inc. (Paris, KY). Isopropanol and ethanol were from ACROS Organics (Geel, Belgium). Acetonitrile was from Alfa Aesar (Ward Hill, MA). 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and Fmoc-glycine (Fmoc-Gly) were from Advanced ChemTech Inc. (Louisville, KY). Finally, water was purified using a NANOPure ultrapure filtration system Barnstead. (Dubuque, IA).

**Equipment:** All reactions were performed inside an FCSII flow chamber Biopetechs Inc. (Butler, PA). Patterning and deprotection were done using light from a mode-locked Tsunami Ti:sapphire laser pumped by a 5 W Millennia Vs diode-pumped cw laser, Spectra-Physics Inc. (Mountain View, CA), through a 20x 0.5NA objective attached to an Eclipse TE2000-U microscope, Nikon Inc. (Japan) equipped with a ProScan microscope stage, Prior Scientific Inc. (Rockland, MA). The laser beam was modulated using a Model 350-80 electro-optic light modulator with model 302 power supply, Conoptics Inc. (Danbury, CT) controlled by software developed in-house. Laser power was measured using a Model 1815-C power meter, Newport Co. (Irvine, CA). Images taken using Cascade Photometrics CCD, Roper Scientific Inc. (Tucson, AZ) through 10x 0.3NA objective lens, Nikon Inc. (Japan) using MetaVue 6.0 software, Universal Imaging Corporation Limited (Marlow, UK) for acquisition and analysis. Scanning electron microscopy (SEM) was performed using a XL30ESEM environmental SEM, FEI Co. (Hillsboro, OR) on a sample coated with 3.5nm palladium /gold.

**Surface Functionalization.** Glass cover slides for a FCSII flow chamber were cleaned using a modification of methods reported by McGall [23]. Briefly: slides were soaked 15 min at RT with 60/40 (v/v) sulfuric acid/hydrogen peroxide (use extreme caution when using this solution), placed in 10% sodium hydroxide (w/v) at 70°C for 3 min and placed in 1% HCl at RT for 1 min. Between each step the slides were soaked in nanopure water for 3 minutes. A solution of 1% 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5 % water was mixed for 10 minutes, and the slides were reacted at RT for 15 minutes with gentle agitation. Slides were then soaked in isopropyl alcohol for 3 min, nanopure water for 1 min, and then placed in a 100°C oven for 5 minutes after which the oven was turned off and nitrogen was blown through for 1 hr. The slides were stored under nitrogen until they were used.

## Light Directed Movement of Polymer Microstructures

**Fabrication of Polymer Structures.** A total of 6mg of AIBN was dissolved in 95  $\mu\text{L}$  HEMA and 579  $\mu\text{L}$  TRIM. This was placed in an optical chamber, and irradiated with 4 mW (all powers reported are measured entering microscope) of 365nm (8nm full-width-at-half-maximum) light for 1.6s per feature through a 20x objective focused 400  $\mu\text{m}$  above the surface of the cover slip. Excess monomer was drained and sample washed with methanol and DMF. The features were spaced 600  $\mu\text{m}$  apart..

**Amination of Microstructures.** Fmoc-Gly was coupled to the photopolymer hydroxyl group using 18.6mg Fmoc-Gly, 22.5mg HBTU, 11.5  $\mu\text{L}$  DIPEA, and 600ul DMF.

Reaction was mixed at 50°C for 30 min. The structures were then rinsed with DMF and the Fmoc removed with 20% piperidine in DMF for 10min. The yield of the reaction was determined using the absorbance at 301nm for the Fmoc-piperidine adduct. Typical polymer substitution levels were 0.1 nanomoles/feature.

**Coupling NVOC and 6-nitrophenyl chloroformate (NPC) to aminated microstructures.** A solution of 19mg NVOC or 14mg NPC, 40 $\mu\text{L}$  DIPEA, and 600 $\mu\text{L}$  DMF was reacted with polymer microstructures by mixing for 30min at 50°C.

**Laser cleavage of NVOC:** The same laser beam used for making the microstructures was used for cleavage of the NVOC. The beam was attenuated as needed.

Swelling and Tip Velocity Measurements: Images taken of the microstructures at the glass/polymer interface in various solvents were manually fitted with ellipses of known pixel area. Tip velocity was calculated from the distance moved in sequential images over a known amount of time.

## Results and Discussion

**Porous polymer microstructures.** Polymer structures were obtained via the photopolymerization of HEMA and TRIM with AIBN. Oxygen quenching[6] and light

**Light Directed Movement of Polymer Microstructures**

attenuation from AIBN absorption were used to limit the polymer structure dimensions to the volume of excitation between the surface and the focus of the laser.

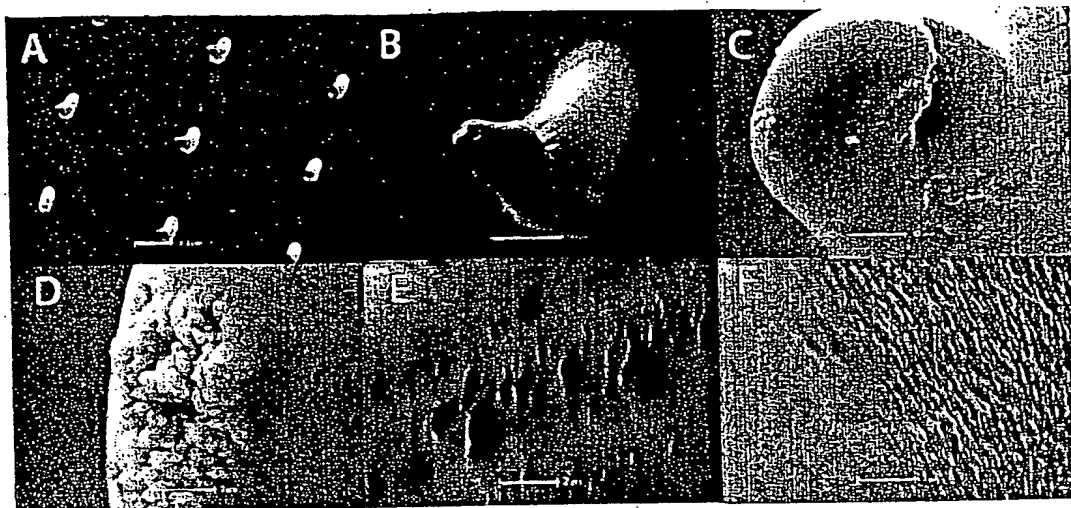


Figure 1. SEM images of polymer microstructures: (A) a portion of the array, (B) one microstructure, (C) top of microstructure, (D) macropore at tip of microstructure, (E) macropores below tip, (F) junction of smooth central region with lowest rough/microporous region.

An array of polymer features was generated by laser-directed photopolymerization (Figure 1A). Partial polymerization results in soft porous structures that were measured on the optical microscope to be 400  $\mu\text{m}$  tall, having an elliptical base with radii of 75 and 200  $\mu\text{m}$ . The structure of each feature has a heterogeneous morphology due to spatial differences in light intensity in the focused laser beam. Structures appear composed of four regions (Figure 1B): two macroporous regions close to the beam focus (Figure 1C-E), an apparently nonporous central region (smooth area in Figure 1F), and a rough potentially porous region nearest the glass surface (rough area in Figure 1F). The macropores at the top are on order of 1  $\mu\text{m}$ . The number of reactive sites (using FMOC as a probe) was estimated to be 0.1 nmole per feature, 5 orders of magnitude more than would be expected for a nonporous material.

**Solvent Swelling:** The swelling of the polymer with and without the NVOC protective group was measured in various solvents. It was found that the greatest swelling, and largest difference in swelling between the protected and unprotected resin, was in the polar aprotic solvents DMF and acetonitrile (Figure 2). The swelling of the polymer was found, as expected, to be related to

Light Directed Movement of Polymer Microstructures

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the Hildebrand solubility parameter [24] where maximum swelling of a slightly crosslinked polymer occurs in solvents of similar solubility parameter [24].

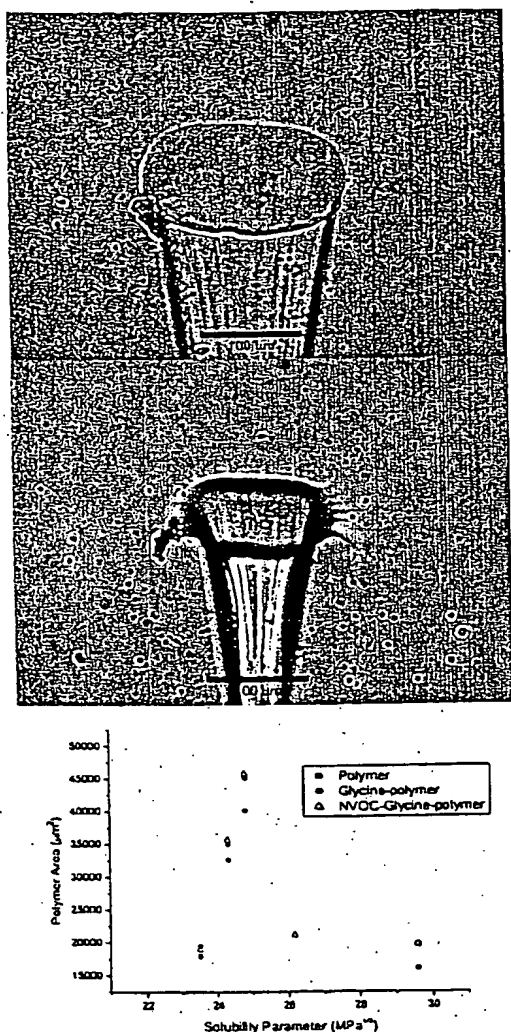


Figure 2. (top) Images of NVOC-Gly polymer features laying on the glass surface, illustrating the difference in swelling when exposed good solvents (acetonitrile) and poor solvents (methanol). The images were recorded using a 10x objective lens focused at polymer glass  
Light Directed Movement of Polymer Microstructures

interface. (bottom) swelling of polymer, Glycine-polymer, NVOC-Gly polymer in various solvents as a function of the Hildebrand parameter.

It is apparent from Figure 2 that the swelling of the resin changes dramatically with the solvent. In DMF the area of the NVOC-Gly polymer at the glass polymer interface increased 10% versus the Gly polymer. This has been seen before; it was found by Merrifield that over the course of solid phase peptide synthesis, resin swelling increased more than five fold [25]. This behavior was attributed to the net decrease in free energy upon swelling due to solvation of peptide chains bound to the polymer matrix. Presumably a similar solvation mechanism accounts for the differential swelling of the resin with and without NVOC as shown in figure 2.

**Polymer Movement.** Upon laser excitation of the NVOC-Gly polymer structures in DMF or acetonitrile, the NVOC is photocleaved, resulting in shrinking of the illuminated portion of the polymer, causing the polymer to bend. Figure 3 shows a series of images collected as a polymer structure moves towards the laser beam.

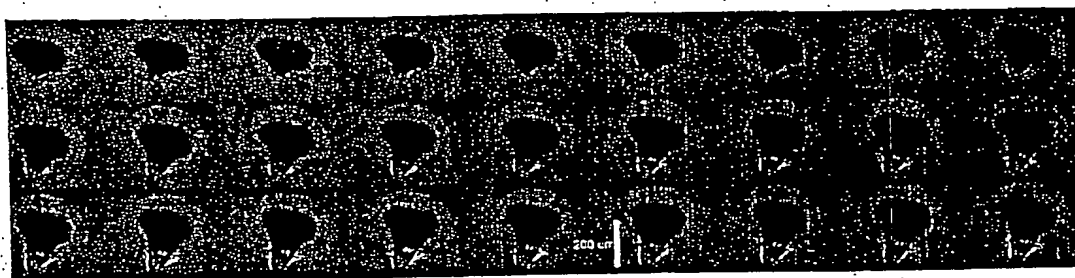
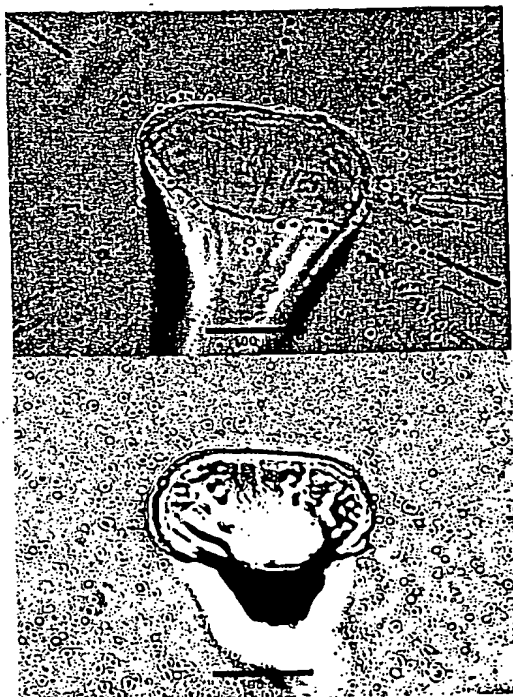


Figure 3. Progression of images of NVOC-Gly polymer structure with asymmetric illumination at lower edge of polymer structure. Perspective is looking down on the polymer as it bends toward the lower edge of the images. The upper left image of the series represents the point at which illumination was initiated, with time continuing from left to right and then down the array. Images were taken through a 10x objective lens at 5ms per frame and the solvent used was acetonitrile.

#### Light Directed Movement of Polymer Microstructures

Symmetric illumination of an NVOC-Gly polymer feature results in the rapid release of solvent from the microstructure. Small particulates in solution rapidly move radially away from the polymer structure during shrinkage with a maximum velocity of 0.01 mm/s. This provides a lower limit for the maximum fluid velocity. It should also be noted that the NVOC group cleaves as nitrosobenzyl aldehyde [Patchornik, 1970 #1] which is released upon illumination. A modified photocleavable group of this nature could be used as a method for local delivery of reagents or drugs.

The base area of the polymer shrinks by ~4% after a 20sec 400uW illumination period (Figure 4 top and bottom respectively). Given that the polymer is covalently attached to the glass it is expected that the actual bulk shrinkage is greater than is observed at or near the glass surface.



Light Directed Movement of Polymer Microstructures

Figure 4. (top) Movement of particles away from polymer upon illumination with light.

(bottom) Difference image showing shrinkage of polymer structure upon laser excitation before (black) and after (white) 20sec of 400  $\mu$ W

The polymer movement is very rapid especially at the tip of the polymer structure. Velocities on order of 1mm/sec were recorded (Figure 5) in acetonitrile. This is several times faster than that observed in DMF (Figure 5) even though the differential swelling is larger in DMF (Figure 1). This is explained by the three fold higher viscosity of DMF resulting in greater resistance to flow, slowing the movement of the microstructure. It is also clear that there was little if any movement of structures in solvents that did not swell the resin (Figure 5).

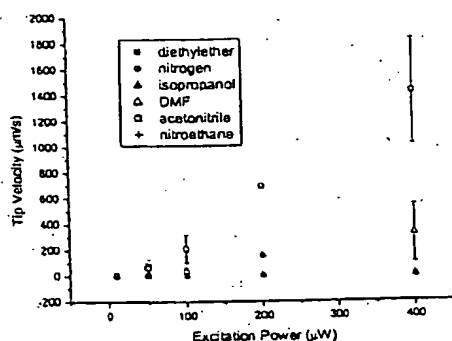


Figure 5. Tip velocity as a function of excitation power in various solvents.

Chemical mechanism of polymer volume change upon illumination: The design hypothesis

underlying the development of this system was that photocleavage induced changes in surface chemistry result in the volume changes and associated polymer movements. The other possible mechanisms for mechanical movement upon illumination and photocleavage include photochemical curing processes, electrostatic forces, hydrogen bonding, and optical trapping, and measurements were performed to investigate each of these.

A photochemical curing process is unlikely for several reasons. The polymer movement continues for ~1sec after a 100ms 1mW exposure in DMF. Given the presence of oxygen and low viscosities of the solvents it is unlikely that any free radical or photochemical process would continue for this long in the dark. It was also found (Figure 6) that these dramatic movements

#### Light Directed Movement of Polymer Microstructures

only occurred with NVOC and not in the presence of the polymer itself or with another chromophore (NPC). It was possible to reattach NVOC and regain partial polymer movement.

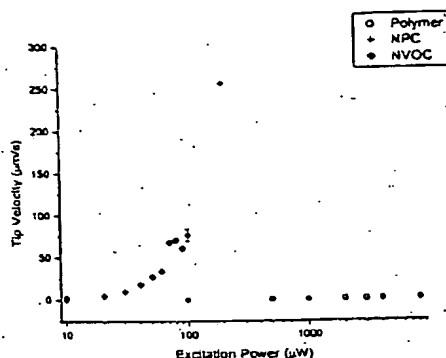


Figure 6. Tip velocity as a function of input power for polymer structures in DMF.

To test for the role of hydrogen bonding of the hydroxyl or amine groups, the polymer was soaked for approximately 1 hour in acetic anhydride and then illuminated. While this reduced the speed of tip movement (50  $\mu\text{m/s}$  with 400  $\mu\text{W}$  excitation), the movement was still substantial, showing that the movement is not a result of hydrogen bond formation upon NVOC cleavage since all hydroxyls and amines should have been rapidly acylated in acetic anhydride.

The fact that the polymer curves towards the laser beam makes an electrostatic mechanism (formation of protonated amine groups or charged intermediates in the photocleavage reaction) unlikely since one would expect repulsive electrostatic forces to push the polymer away from the illuminated region (Figure 3). Additionally, the movement was not observed in nonpolar solvents, where the electrostatic effect should be greatest and was observed even in a solvent system with a 60 mM ionic strength that should strongly shield the charge-charge interactions (data not shown). Finally, the movement of the polymer is not dependent on the position of the focus, making optical trapping a very unlikely mechanism. Polymer movement is observed even when the focus is at the polymer glass interface, 400  $\mu\text{m}$  from the polymer tip.

## Conclusion

We have described porous polymer structures that make dramatic movements with the rapid release of solvent when illuminated. We hope to attach spiropyrans to these polymer structures in attempt to make a reversible system. This may find use in systems where it is desirable to control the movement of a polymer structure or for releasing material into solution. Such a system would be very exciting for converting light energy into mechanical movement.

## Light Directed Movement of Polymer Microstructures

## Acknowledgements

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Center for Solid State Science at Arizona State University for SEM

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Light Directed Movement of Polymer Microstructures

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25. Sarin, V.K., S.B.H. Kent, and R.B. Merrifield, *Properties of Swollen Polymer Networks - Solvation and Swelling of Peptide-Containing Resins in Solid-Phase Peptide-Synthesis*. Journal of the American Chemical Society, 1980. 102(17): p. 5463-5470.

This invention can be used as \_ This technology would allow the conversion of light energy to mechanical energy either through the movement of a fluid or the polymer itself. There would seem to be a wide range of possibilities for such a material. Ranging from large solar collectors, light powered nanobots (photopolymer structures 100's of nanometers can be readily made), artificial muscle, drug delivery systems, microfluidic pumps and valves, etc.

1. This invention provides the following advantages: It results in dramatic changes in the physical dimensions of the polymer.
2. It releases (or could absorb) solvent
3. It is a general system that could be used with any porous polymer formulation or potentially on the surface of very thin polymer structures.
4. The surface area of a porous polymer is many orders of magnitude higher than a non porous polymer, this would be the preferred mode for drug delivery

There are molecules (Azobenzene, spiropyrans, etc) that act as molecular switches, one color of light puts them in one form, another moves them back to the initial form. By attaching one of these molecules to the surface that has a large polarity change upon switching forms, it should be possible to make a polymer that expands with one color of light and contracts with another color of light. This technology would allow the conversion of light energy to mechanical energy either through the movement of a fluid or the polymer itself. There would seem to be a wide range of possibilities for such a material. Ranging macroscale solar collectors, light powered nanobots (photopolymer structures 100's of nanometers can be readily made), artificial muscle, drug delivery systems, microfluidic pumps and valves, etc.

There is a NVOC derivative that is used as a linker in peptide synthesis. It could be used to release a material of interest. One end of the linker would be linked to the polymer, the other to the material (drug) to be released. By adjusting the surface energy of the polymer it would be possible to design a system that would rapidly release the material with light. Merrifield has shown that polymer resin swells 5x with a large peptide attached—this system with a photocleavable linker would allow the delivery of peptide (and other) drugs accompanied with a rapid movement of solvent.

It may be possible to use a conducting polymer and switch the polymer states by oxidizing and reducing groups on the surface electrically. This would allow this technology to be used in places not accessible to light (inside the body) or in electrical devices.

There are other photoactivated groups and polymers that could be used.

# Document made available under the Patent Cooperation Treaty (PCT)

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Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1331930

# THE UNITED STATES OF AMERICA

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*June 09, 2005*

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APPLICATION NUMBER: 60/623,181

FILING DATE: *October 29, 2004*

RELATED PCT APPLICATION NUMBER: *PCT/US05/15764*



Certified by

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102904

16138 U.S. PTO

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**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

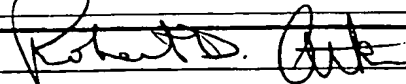
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19249 U.S. PTO  
60/623181

102904

INVENTOR(S)		
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)
Trent Russell Neal Walter	Northen Woodbury	Tempe, Arizona Tempe, Arizona
Additional inventors are being named on the _____ separately numbered sheets attached hereto		
TITLE OF THE INVENTION (500 characters max):		
PEPTIDE CHARACTERIZED FOR PATTERNED PHOTOPOLYMER FORMED USING LIGHT DIRECTED SYNTHESIS		
Direct all correspondence to: CORRESPONDENCE ADDRESS		
<input checked="" type="checkbox"/> The address corresponding to Customer Number: 26707		
OR		
<input type="checkbox"/> Firm or Individual Name		
Address		
City	State	Zip
Country	Telephone	Fax
ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Specification Number of Pages 21		
<input type="checkbox"/> Drawing(s) Number of Sheets		
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		
<input type="checkbox"/> CD(s), Number of CDs		
<input checked="" type="checkbox"/> Other (specify) Cover Sheet; postcard		
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT		
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.		
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.		
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.		
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055		
A duplicative copy of this form is enclosed for fee processing.		
<input checked="" type="checkbox"/> invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.		
<input type="checkbox"/> No.		
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____		

SIGNATURE



Date October 29, 2004

TYPED or PRINTED NAME Robert D. Atkins

REGISTRATION NO. 34,288

TELEPHONE 602-229-5311

(if appropriate)  
Docket Number: 112624.00138**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

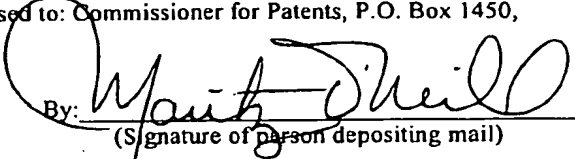
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1882295

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Date of Signature  
and Deposit: October 29, 2004

By:   
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MARITZA O'NEILL

**CERTIFICATE OF MAILING PURSUANT TO 37 C.F.R. 1.10**

Applicant: Trent R. Northen *et al.*

Date of Filing: October 29, 2004

Title: PEPTIDE CHARACTERIZED FOR  
PATTERNED PHOTOPOLYMER  
FORMED USING LIGHT DIRECTED  
SYNTHESIS

Art Unit: Unassigned

Examiner: Unassigned

Attorney Docket No.: 112624.00138

Commissioner for Patents  
P.O. Box 1450  
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Type of Filing:

- 1) Provisional Application For Patent Cover Sheet
- 2) Specification (21 pages, plus cover sheet)
- 3) Return postcard

**PATENT**

**PROVISIONAL APPLICATION**

**of**

**TRENT RUSSELL NORTEN  
NEAL WALTER WOODBURY**

**For**

**UNITED STATES LETTERS PATENT**

**on**

**PEPTIDE CHARACTERIZED FOR PATTERNED PHOTOPOLYMER FORMED  
USING LIGHT DIRECTED SYNTHESIS**

**Attorneys:**

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PHOENIX, AZ 85004-2391**

**Express Mail Label No.: EL988555877US  
Attorney Docket No.: 112624.00138**

**BEST AVAILABLE COPY**

Approach: to pattern Fmoc-G6FL C<sub>60</sub>H<sub>18</sub> on the polymer  
that represents the micro pattern  
P.S.U.  
Hydroxide Antibody I detect via fluorescence.

From page 29 9.5 mL Hexane + 57.2 mL Toluene + 4 mg ABN.  
Used instead of Sonicated until dissolved  
added to chamber of smooth methacrylated slide  
as noted on page 29 200 730/2 8mFWHM 8mW 2ke 525nm  
2x 27x13 arrays, looks great!

to use Fmoc-G6FL C<sub>60</sub>H<sub>18</sub> prep. by RAN. Still  
dissolved in TFA/THF Supersol solution. 1.2 mL - 1.1 = 80 mg  
extracted w/ MeOH (4x) kept in (60 mL) DIPEA in  
meOH unreacting. Dried down from liquid (yellow)  
Bong - 60 mg (7.42 mg) = 35.5 mg peptide  
Fmoc-G-G-F-L C<sub>60</sub>H<sub>18</sub>

$$\text{Fmoc } 223.3 + 2(57.05) + 147.18 + 113.16 + 17 = 614.74 \frac{\text{g}}{\text{mole}}$$

$$35.5 \text{ mg} / 614.74 \frac{\text{g}}{\text{mole}} = 0.058 \text{ mmole} \times \left( \frac{2.35}{2.5} \right) = 5.5 \times 10^{-2} \text{ mmole DIPEA}$$

$$(5.5 \times 10^{-2} \text{ mmole}) (379.25 \frac{\text{g}}{\text{mole}}) = 20.9 \text{ mg HBTU}$$

$$0.29 \text{ mmole} (127.25 \frac{\text{g}}{\text{mole}}) (1.4 \frac{\text{mL}}{\text{mg}}) = 52.5 \mu\text{L}$$

$$\frac{30 \text{ mM}}{1000 \text{ mL}} (614.74 \frac{\text{g}}{\text{mole}}) = 9.22 \text{ mg } 9.5 \text{ mg peptide}$$

$$(30 \text{ mmole}) (379.25 \frac{\text{g}}{\text{mole}}) = 5.68 \text{ mg HBTU}$$

$$(60 \text{ mmole}) (127.27 \frac{\text{g}}{\text{mole}}) (7.42 \text{ mg}) / 5.6 \text{ mL DIPEA}$$

use 150 mM MeOH Read and Understood By  
2-15-04 JF Mar 13. mL

Signed

Date

Signed

Date

10/29/04

Followed procedure from T-8 - 71

Patterning looks great

- ① Rinsed 30 min w/ 2x DMF
- ② Wash/HSTU/DMF 11:00 am - 12:04 pm 50°C 150 Rpm
- ③ 1hr @ 50°C DMF, 3x 2min 10min DMF  $\text{OP}_{301} = 0.053$
- ④ 10 min procedure  $\text{OP}_{301} = 4 \times 1.24 = 4.46$
- ⑤ Rinsed 4x DMF
- ⑥ Fmoc YGG-L-COOH ~ 15% / 13% DMF / 5.4% HSTU / 60% DMF
- ⑦ 20 min 9:29 am 50°C 150 Rpm
- ⑧ 4pm Rinsed 2x @ 50°C 2x (2x DMF)
- ⑨ 10min DMF  $\text{OP}_{301} = 0.078$
- ⑩ 10min procedure  $\text{OP}_{301} = 1.7$
- ⑪ 3x DMF
- ⑫ 14mg Fmoc YGG-L-COOH 60% DMF 6pm 150 Rpm 50°C
- ⑬ Rinsed 3x DMF 7pm left in DMF overnight
- ⑭ 8:30 pm 7/30 Rinsed 3x DMF 3x ACN
- ⑮ Added 60% ACN + 20% DMF 1/20
- Later as before 7:30/2 200 Rpm
- adjust to 200 Rpm w/ 200 20 sec exposure
- for lower energy
- It was observed that the resin no longer swelled when in Acetonitrile, -1 200 Rpm 20 sec exposure to elute w/ ACN 70823
- washed 3x w/ DMF ACN DMF ACN  $\text{AREA} = 150471$
- washed 2x. reference
- ⑯ Rinsed 3x DMF ACN 3x DMF, 3x ACN
- Dried w/  $N_2$

Continued on Page \_\_\_\_\_

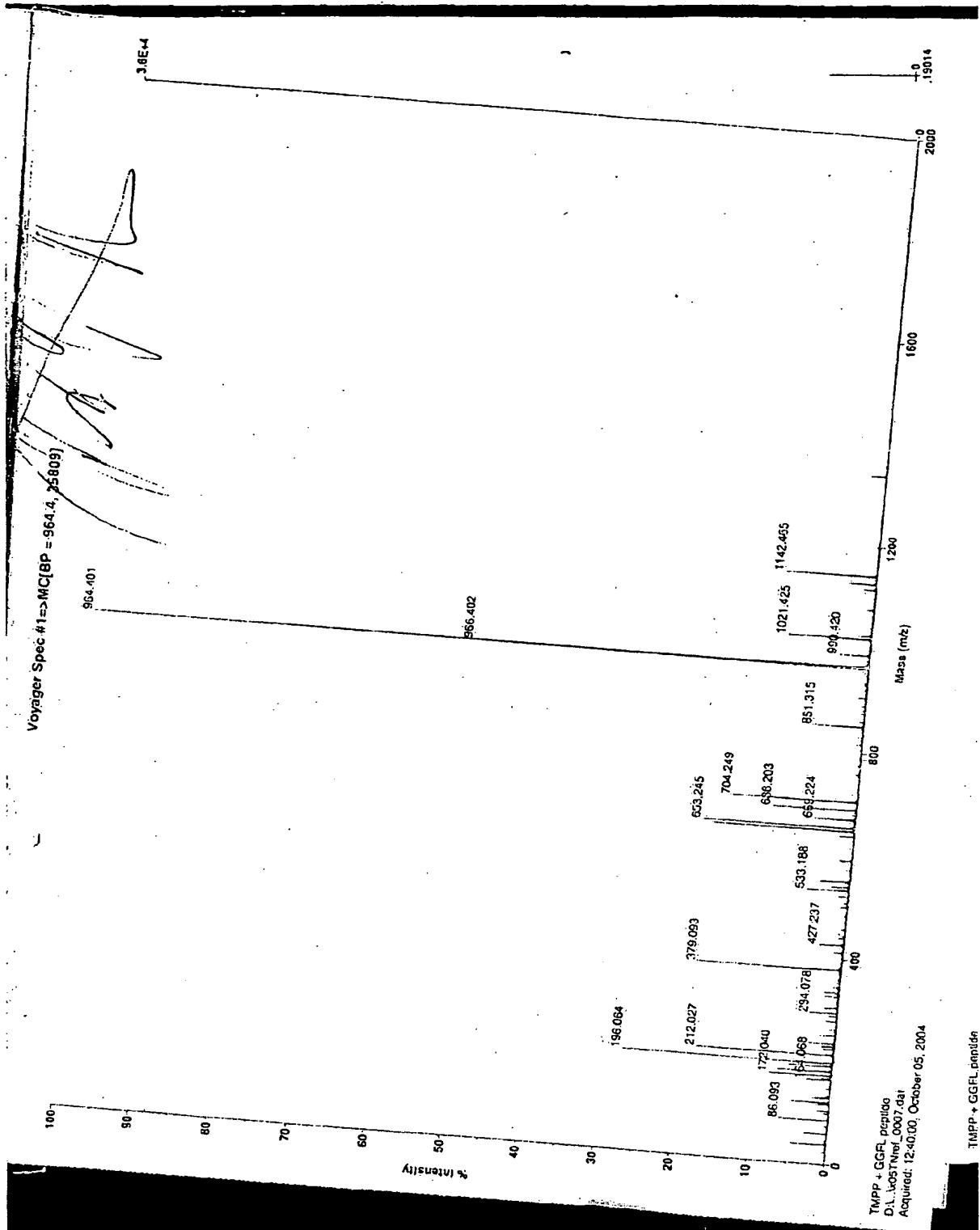
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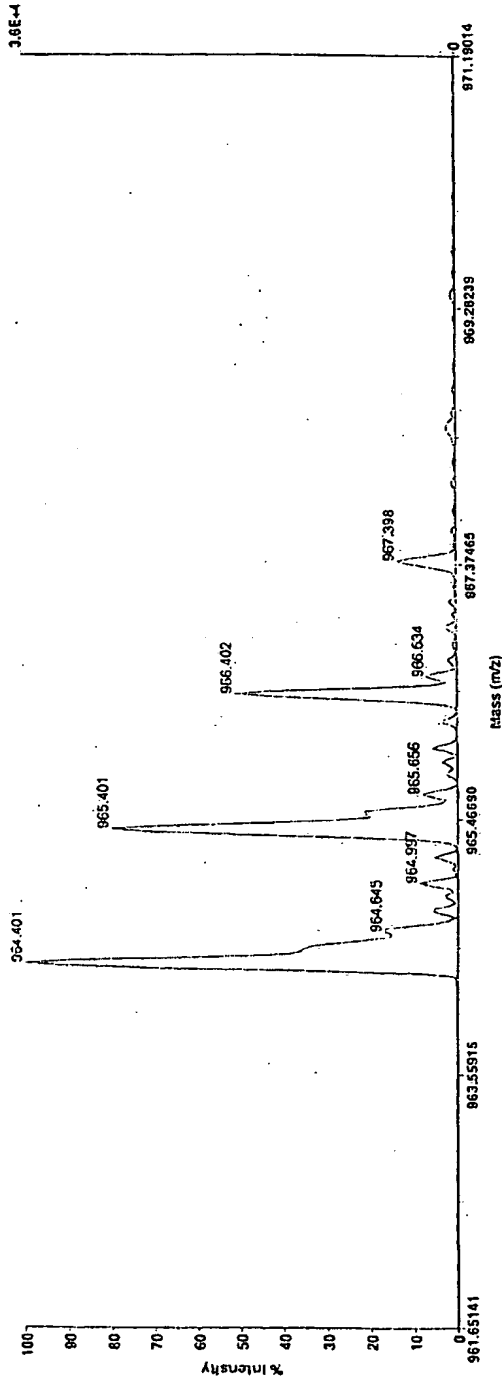
Date

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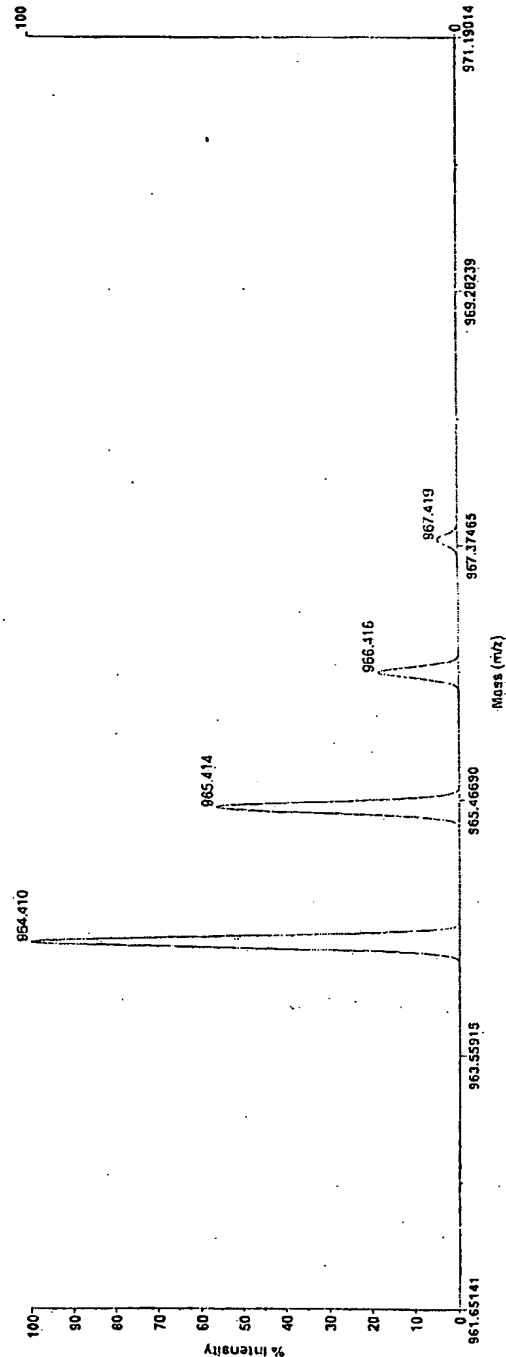
Date



Voyager Spec #1=>MC(BP = 964.4, 35809)

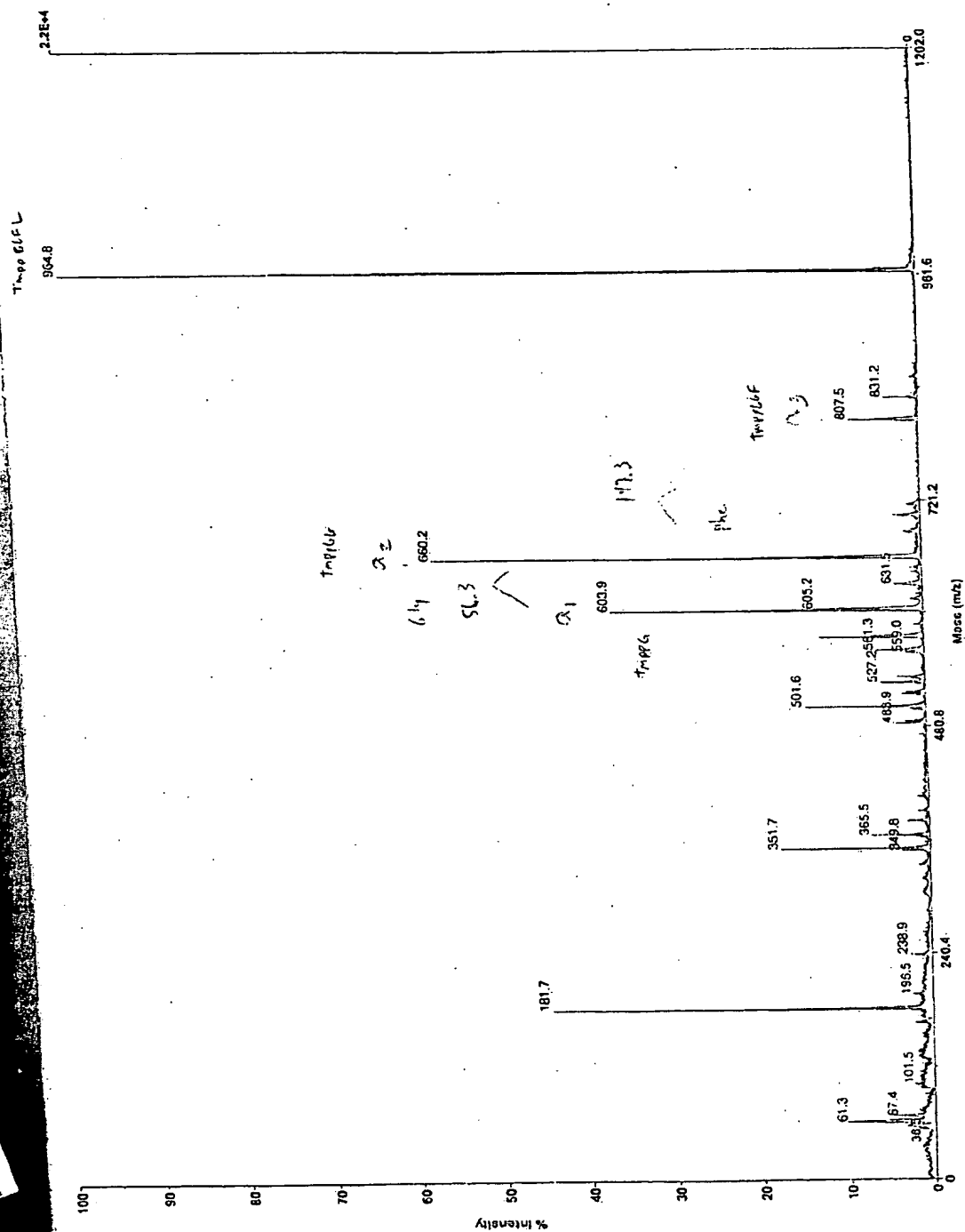


ISO-C48H63N5O14P



TMPP + GGFL peptide  
D:\\_VOSTNref\_0007.dat  
Acquired: 12:40:00, October 05, 2004

TMPP + GGFL peptide  
D:\\_VOSTNref\_0007.dat  
Acquired: 12:40:00, October 05, 2004



GRGDSP

PROJECT

Notebook No. \_\_\_\_\_

85

Continued From Page \_\_\_\_\_



(1.0 mg (0.02 mL)) (76.8 mg) = 0.0768 mg Temp-Ac - 0.5 Jt Br<sup>-</sup>

from D. Brown Lab. Analytical Section  
#68 305-317 1999

5x = 0.384 mg

~ 0.5 mg Temp-Ac - 0.5 Jt Br<sup>-</sup> + 20 mL H<sub>2</sub>O + 480 mL DMF  
10 on shelf @ 35°C 150 RPM

② 11 to 20 added 30 DMF left @ 150 RPM 35°C - 1 pm  
Added 3x more! Removed 1/2 of each array  
placed in refrigerator w/ 100% 1,2 TFE to prep. S. line / TFA  
Sonicated for 15 min. Initial exposed sound to 5.11  
apart more than in exposed.

Unsubstantiated! Come to find

Mass 5-mg clearly shows the Temp-66FL more C<sub>13</sub>H<sub>18</sub>N<sub>5</sub>O<sub>14</sub>P 946.401  
There is the correct 119.002 pattern: The post source decay  
shows Temp-66FL, Temp-66F, Temp-66, Temp-6  
- this was successful. There was a small amount of Temp-66FL  
in the Control, however. The major product was the 653.24 1.0  
corresponding to mass-66FL. This demonstrates the  
successful light directed synthesis of a product on a photo-  
patterned polymer support & the mass spec characterized  
of the product from the polymer material. It may be  
possible to do the mass spec in situ by spraying matrix/TFA  
directly on the plate (lower) - looking into this.

Read and Understood By

Signed

10.27.04

Date

Signed

10/29/04

Date

9E

30821

PROJECT \_\_\_\_\_

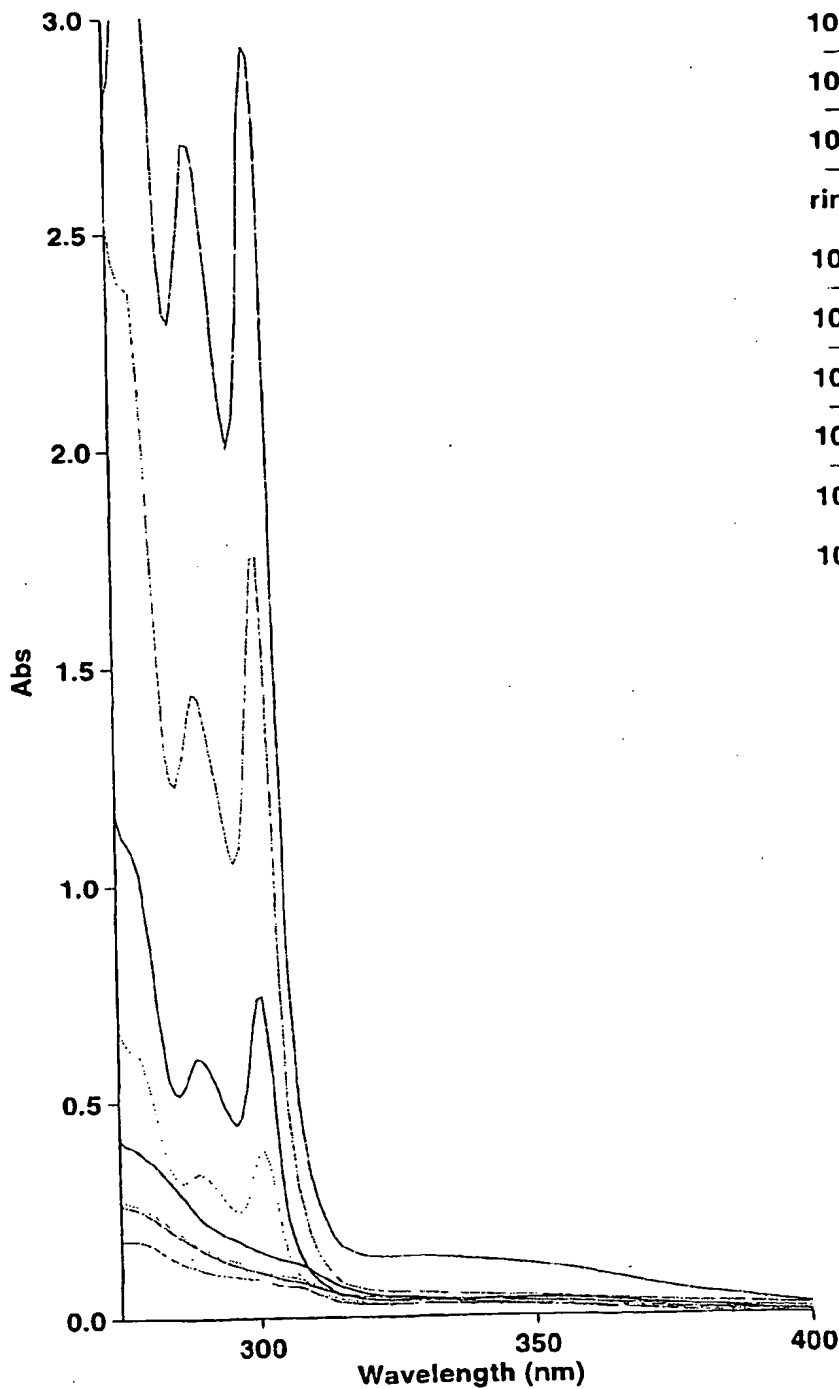
Notebook No. \_\_\_\_\_

Continued From Page \_\_\_\_\_

72<sup>73</sup>

9/20/2004 1:25:05 PM

Page 1 of 24



- 10min rinse2
- 10min piperidine
- 10min rinse after
- rinse after coupl
- 10min piperidine
- 10 min rinse afte
- 10 min deprotect
- 10 min rinse afte
- 10 min rinse afte
- 10 min piperidin

RT

720  
660  
600  
540  
480  
420  
360  
300  
240  
180  
120  
60  
0

72

Signed \_\_\_\_\_

Date \_\_\_\_\_

Signed \_\_\_\_\_

Date \_\_\_\_\_

10/29/04

Project: to pattern Fmoc-GGFLCock on the polymer  
that represents w/ Nuc & pattern  
P.S.U.  
Hydride Antibody & detect the fluorescence.

Comp-se 21 45ul Hexa + 572ul DMF + 4mg ABN  
Used & Sonicated until dissolved  
added to Chamber w/ Growth methacrylate slide  
patterned at 200 page 29 200 730/2 800FWHM 80W 2sec 525nm  
2x 27x13 arrays, looks great!

Lengths use Fmoc-GGFLCock PRP-10 by EAN. Still  
dissolved in TFA/THF: 50:50:10:10  
extracted w/ MeOH (Ag. knot w/ 60% D.P.P.A. in  
meOH unnecessary) Dried down from layer (yellow)  
Bong - 60ul (742mg) = 35.5mg peptide  
Fmoc - G - G - F - L (6014)

$$\text{Fmoc } 223.3 + 2(57.05) + 147.18 + 113.16 + 17 = 614.74 \frac{\text{mg}}{\text{mole}}$$

$$35.5 \text{ mg} / 614.74 \frac{\text{mg}}{\text{mole}} = 0.058 \text{ mmole} \times \left( \frac{2.35}{2.8} \right) = 5.5 \times 10^{-2} \text{ mmole}$$

$$\times 28 = 0.29 \text{ mmole D.P.P.A.}$$

$$(5.5 \times 10^{-2} \text{ mmole}) (379.25 \frac{\text{mg}}{\text{mole}}) = 20.9 \text{ mg HBTU}$$

$$(0.29 \text{ mmole}) (129.25 \frac{\text{mg}}{\text{mole}}) (1.4 \frac{\mu\text{L}}{\text{mg}}) = 52.5 \mu\text{L}$$

9.15.04

$$(30 \text{ mM}) (614.74 \frac{\text{mg}}{\text{mole}}) = 9.22 \text{ mg}$$

9.5 mg p-ptr

$$(30 \text{ mM}) (379.25 \frac{\text{mg}}{\text{mole}}) = 5.68 \text{ mg HBTU}$$

$$(60 \text{ mM}) (129.25 \frac{\text{mg}}{\text{mole}}) (1.4 \frac{\mu\text{L}}{\text{mg}}) = 5.2 \mu\text{L D.P.P.A.}$$

Use 150 mM MeOH  
9.15.04  
Signed \_\_\_\_\_ Date \_\_\_\_\_  
Read and Understood By \_\_\_\_\_  
Signed \_\_\_\_\_ Date 10/29/04

Compare Proc - Rank? From page 39 (TNT)

33.73 mg Proc - Rank (2.54)

22.5 mg HSTU (2.382)

11.5 mg D.P.A. (2.642)

$\frac{11.5}{2.64} = 2.84$

21.7 mg

+ 0.6 ml DMF

Start rxn @ 2:30 pm 09/15/04 50°C / 150 RPM

3:30 " " Rank (3x DMF 2x more)

10 min Rank @ 150 RPM  $OD_{301} = 0.15$  Too high

Rank @ 50°C in DMF for 10 min DMF 3x  $OD_{301} = 0.1$

Rank after rxn @ 150 RPM  $OD_{301} = 2.9$

Now need to get all the pipette off

10 min @ 50°C 150 RPM Rank 3x: left overnight

in DMF added 3x w/ DMF  $OD_{301} = 0.147$  looks like

10 min Rank after Proc - Rank

Compare before use 1/3 of peptide solution. 27 mg

came out to 29 mg was close to 1/2 of the solution

+ 6 mg HSTU + 13 ml D.P.A. Sonicated 5 min

added to solution @ 9:10 am 9/16/04

@ 50°C : 150 RPM

11:20 pm 9/16/04 Rank 1, mixed 4x w/ DMF

Rank @ 50°C 5 min @ 12:50 pm - 2:25 pm

Rank 3x DMF 2 min Rank @ 50°C Rank 3x DMF

10 min Rank @ RT  $OD_{301} = 0.11$

+ 10 min 2x2 pipette @ RT 150 RPM  $OD_{301} = 1.75$

Rank 3x DMF

+ 19 mg Proc + 40 ml D.P.A. 600 ml DMF

50°C 150 RPM 3:30 9/16/04 Read and Understood By

Continued on Page 73

Signed \_\_\_\_\_

Date

9/16/04

St. Max

Signed

10/29/04

Date

30920

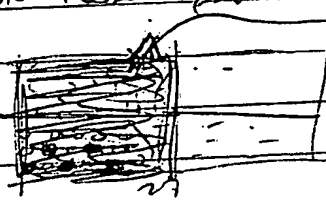
PROJECT

Removal from heat @ 440 pm 1 placed in dish @ rt  
(had been in the dish all along)

⑩ 920 pm Rinsed 3x in DMF ! left overnight  
9/16/04

⑪ washed 3x DMF 10 mM PMSF 0.25% =  
+ 600µl 60mM Sarcosine NL + 20µl Dipep

Experimental Design 1st add protein to external ones  
This will cut any unwanted proteins : block the antibody  
on these antibodies instead



1mJ add gating  
730/2. 8m FWHM  $\sigma = 525\mu$  2x observe

⑫ 1mJ R, Y 1mJ for Y  
0.1mJ for Y  
⑬ 2mJ for Both  
1 See programs  
13 P 720  
12 Y 600  
11 P 600  
10 Y 500  
9 P 400  
8 Y 300  
7 P 200  
6 Y 100  
5 P 50  
4 Y 20  
3 P 10  
2 Y 5  
1 P 0

⑭ 3x DMF, 10 mM DMF, 3x DMF  
+ 20µg Fmoc Phe + 22.5mg HBTU + 21.7µl DiPEA  
50°C 150 rpm 12:07 pm 9.17.04 - 1:10 pm 9.17.04

⑮ Rinsed 3x DMF, 10 mM 50°C 3x DMF, 10 mM DMF

Signed [Signature] Date 9/17/04 Signed [Signature] Date 10/29/04

Read and Understood By  
Continued on Page 79

OD<sub>591</sub> of 10mM Rube = 0.086

① 10mM 2% Rube OD<sub>301</sub> = 0.742 (Scribed or Shunt)

② Rube 300mM

2.9 - 1.75 = 1.15 mV - available to puffer

have removed... my her  
been on 10 mV but not  
recognize

$\frac{0.742}{2.9} = 25.6$  which is the number  
of factors I detected 1 gram

③ 600mM 60mM Sulfonamide + 20mM Rube

1 sec @ 1mV to 0.1mV for 1st 6 sec 9/17/04  
1 sec @ 1mV to 0.1mV for 1st 6 sec (0.1mV)

1 sec @ 1mV to 0.1mV for 1st 6 sec (1mV)

④ 300mM 10mM Rube 30mM Rube

Final yr (Rube) 459.54g/mole

$$\frac{539.6}{33.73} = \frac{459.54}{x}$$

+ 28.73mg Final yr (Rube) + 22.5mg H<sub>2</sub>SO<sub>4</sub> + 21.7mg Rube + 60mM Rube  
437/m 50°C 150 RPM

⑤ Rube 30

+ 60mM 60mM Sulfonamide + 20mM Rube Scribe Rube

Prep test 1/10 (left side) w/ 2mV (1 sec 2mV)  
w/ before.

⑥ Rube 30 10mV @ 50°C

Betaine Monohydrate 94% (Sigma) Pw = 135.2

$$\frac{539.6}{33.7} = \frac{135.2}{x}$$

8.4mg Betaine  
22.5mg H<sub>2</sub>SO<sub>4</sub>  
21.7mg Rube

Will set Betaine to dissolve

Rube 30 w/ part 1  
left @ 5°C over the weekend

Read and Understood By

Continued on Page 75

Signed \_\_\_\_\_

Date

7/17/04

Signed \_\_\_\_\_

Signed

6/29/04

Date



PROJECT

Notebook No. \_\_\_\_\_  
Continued From Page

74 75

9.20.04 Required 100% must to possible = 1.00  
Try 50% must, rather to be sure, it, went  
to minimum reaction error found.

① 500  $\mu$ l DMF + 80  $\mu$ l MeOH + 8.5  $\mu$ g Methane + 21.7  $\mu$ l D<sub>2</sub>O

② + 22.5  $\mu$ l H<sub>2</sub>O

3  $\mu$ l added to cluster reacted @ RT 9.20.04 92.2m

11:20 am 9.20.04 reacted 3x w/ DMF, 1 hr DMF, 10 min DMF

$$OD_{301} = 0.086$$

④ 10  $\mu$ l 2.2% p-phenol

$$OD_{301} = 0.79$$

⑫ reacted 3x DMF 15 min DMF 3x DMF

$$\begin{array}{r} 1 \\ 3 \\ 2 \\ \hline 7.8 \\ \sim 50\% \text{ of} \\ \sim 2 \text{ min} \end{array}$$

Antibody hybridized

3x wash w/ PBS Buffer pH=7.2 30 min

100  $\mu$ l of L-8516 UV-crosslinked Antisense (RT 30.1)

+ 900  $\mu$ l PBS

2:11 pm 9.20.04 - 3:52 pm

TM 9.20.04

Reacted 3x with 0.05% Tween 20/PBS

4 pm 9.20.04 1/1000 Dilution of Antisense

5  $\mu$ l mixed 2x w/ 0.05% Tween 20 in PBS

1000  $\mu$ l 488 nm excitation 75% SL

10  $\mu$ l get the opposite effect I was expect.

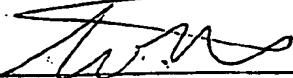
Could be that the antibody binds YP66FL or  
more likely is from the Fluorescent photo product.

Conclusion | The most interesting part so far is that I am  
getting ~ 100% yield @ (2mJ) I say much more

fluorescence which is good for getting close to

100% w/ (2mJ)

Next Read and Understood B&F for notes - ms



Signed

9/12/04

Date



Signed

10/29/04

Date

800

720

640

Mass (m/z)

Sample A: Hatched, 2004, m/z

13

Scraped off the two halves of the paper pattern  
 & placed the polymerized fragments into separate microfuge  
 tubes added 200  $\mu$ l 50:50 TFA in DCM w/ 3% Tri:SO<sub>2</sub>-A/Soln  
 10:55 am 9.21.04

1:30 pm still yellow add mineral oil about 2 drops

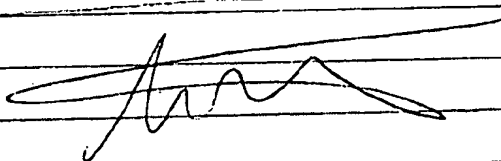
Observation the fibers are yellow, both for the fragment &  
 Control. I hope they will become clear after the cleanup?

did not change in 1:1 DCM:TFA

1:35 pm added more TFA & immediately the  
 fibers lost their color after about  
 minutes added 0.3  $\mu$ l Tri:SO<sub>2</sub>-A/Soln

not there, polymer floats in TFA so it  
 was dispersed so I GULP see the color  
 added 100  $\mu$ l acetonitrile & color less color

And down a glass to one B in 5

 9/21/04

1 hour 9/23/04

Repeated cleanup and 2 hours of B-66 PL mass  
 in one new NDC-66 PL-mass

in 12 TFA-saturated Soln in TFA

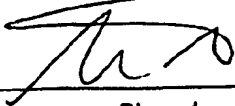
clear brown array (66 PL & YPL 6 PL) in 1:1

TFA = Acetonitrile w/ 0.5% Tri:SO<sub>2</sub>-A/Soln same  
 when polymer may have fallen apart.

MALDI-MS shows m/z = 440.32 peak which is 100 off from predicted polymer  
 structure Control shows m/z = 653.259. NDC 66 PL-mass  
 10/29/04

Continued on Page

Read and Understood By



Signed

9.22.04

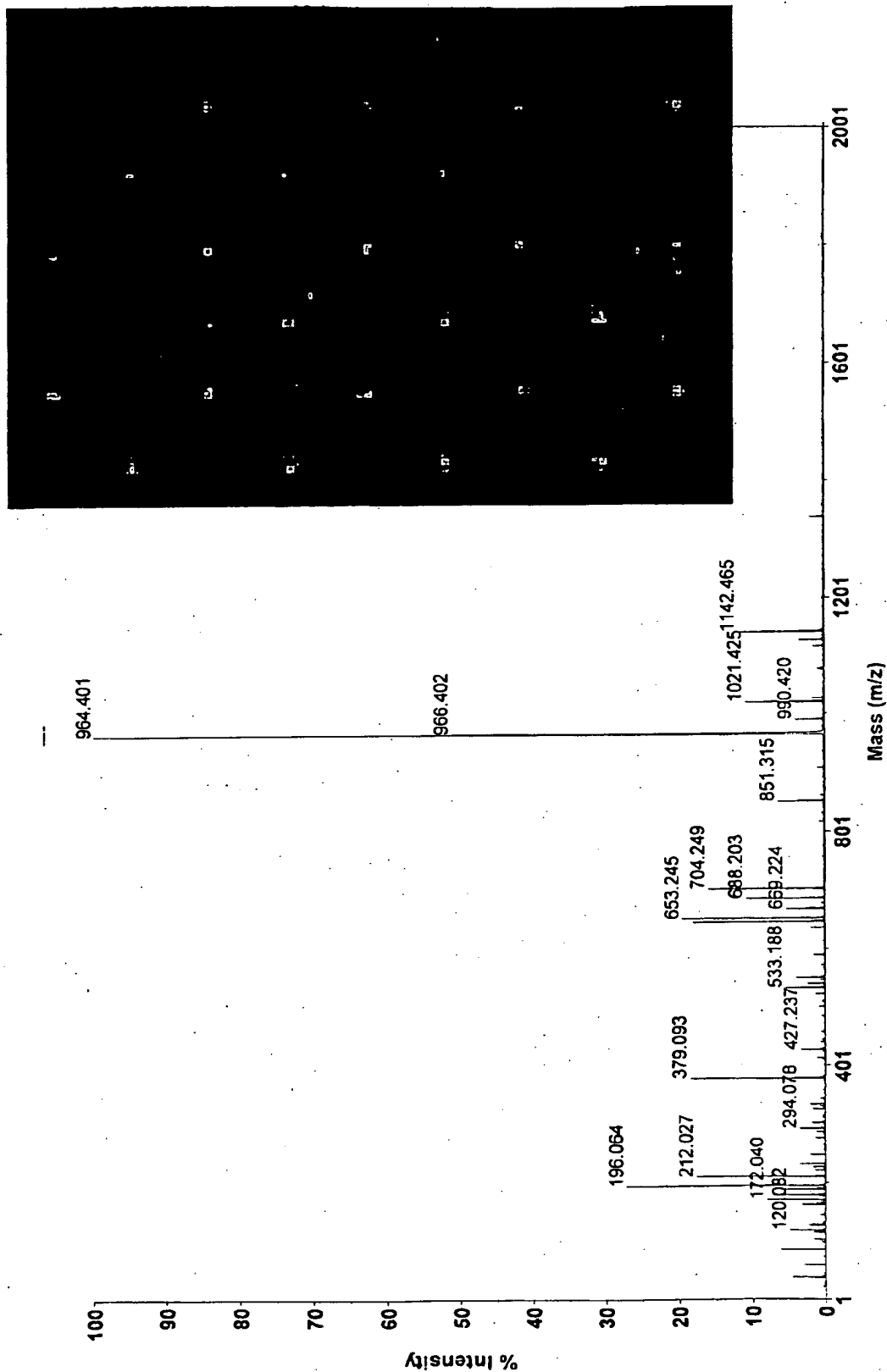
Date



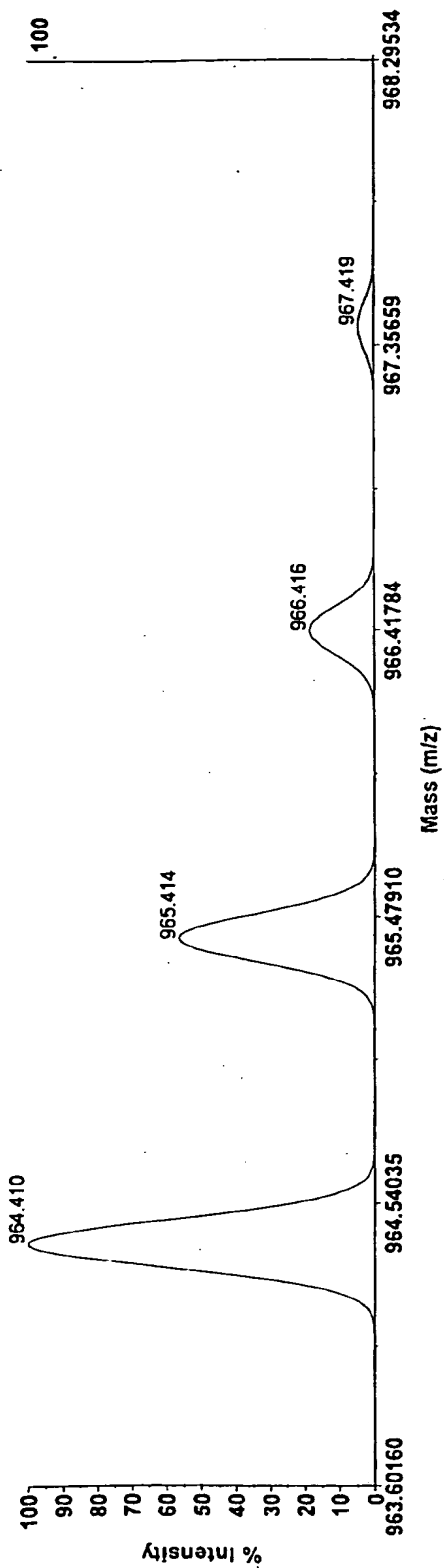
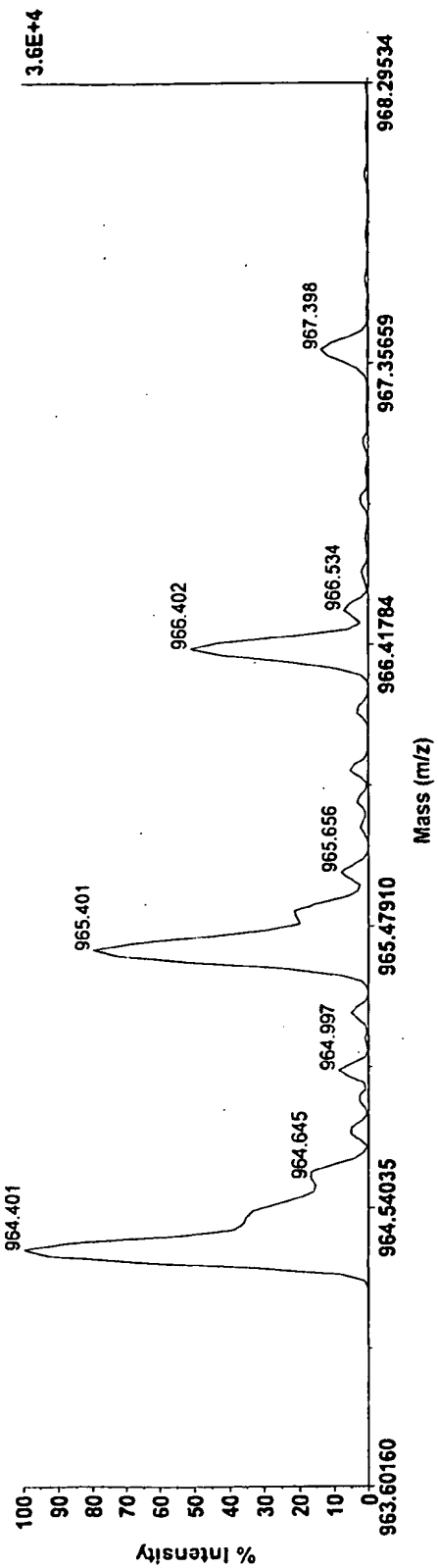
Signed

10/29/04

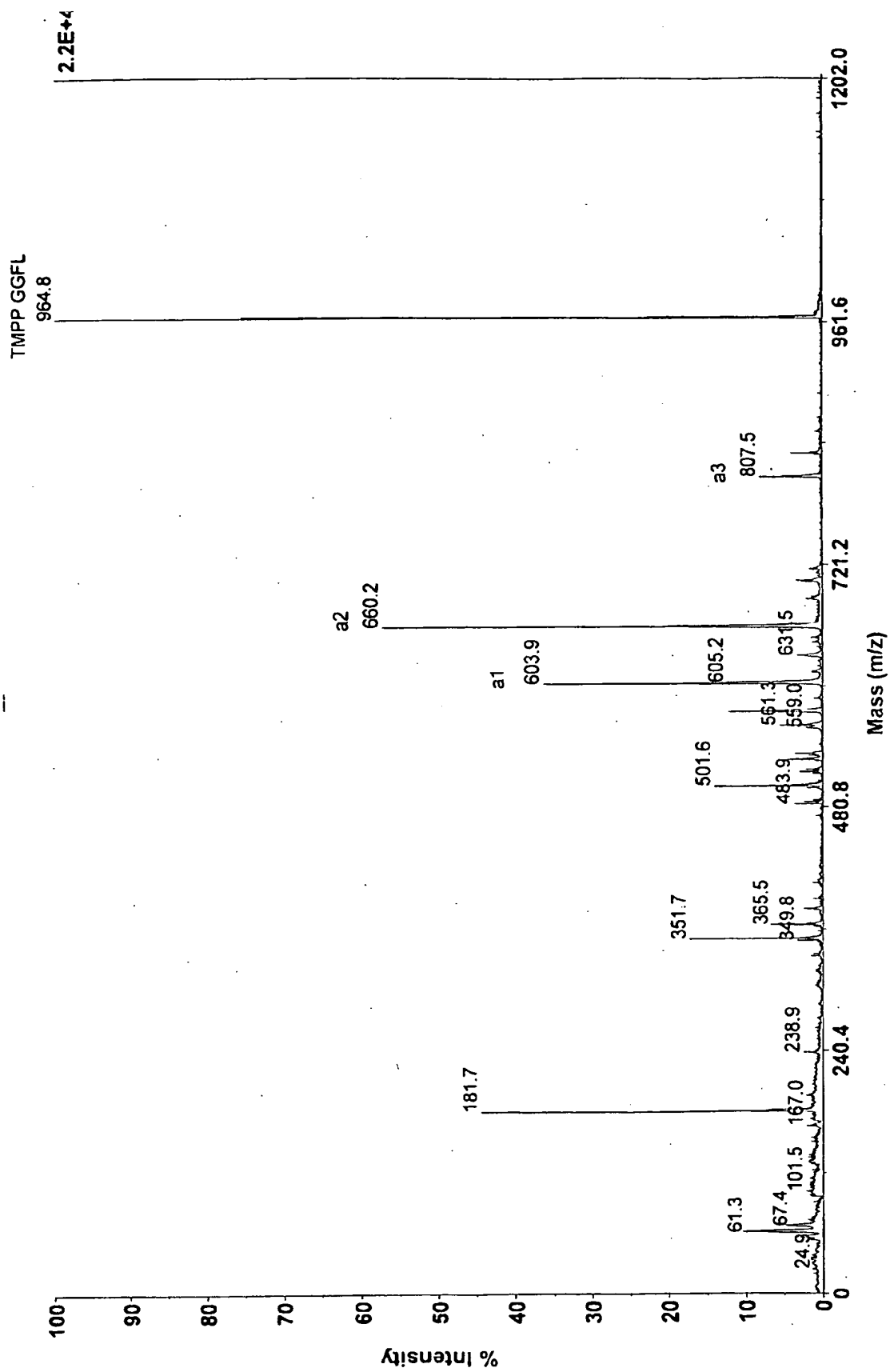
Date



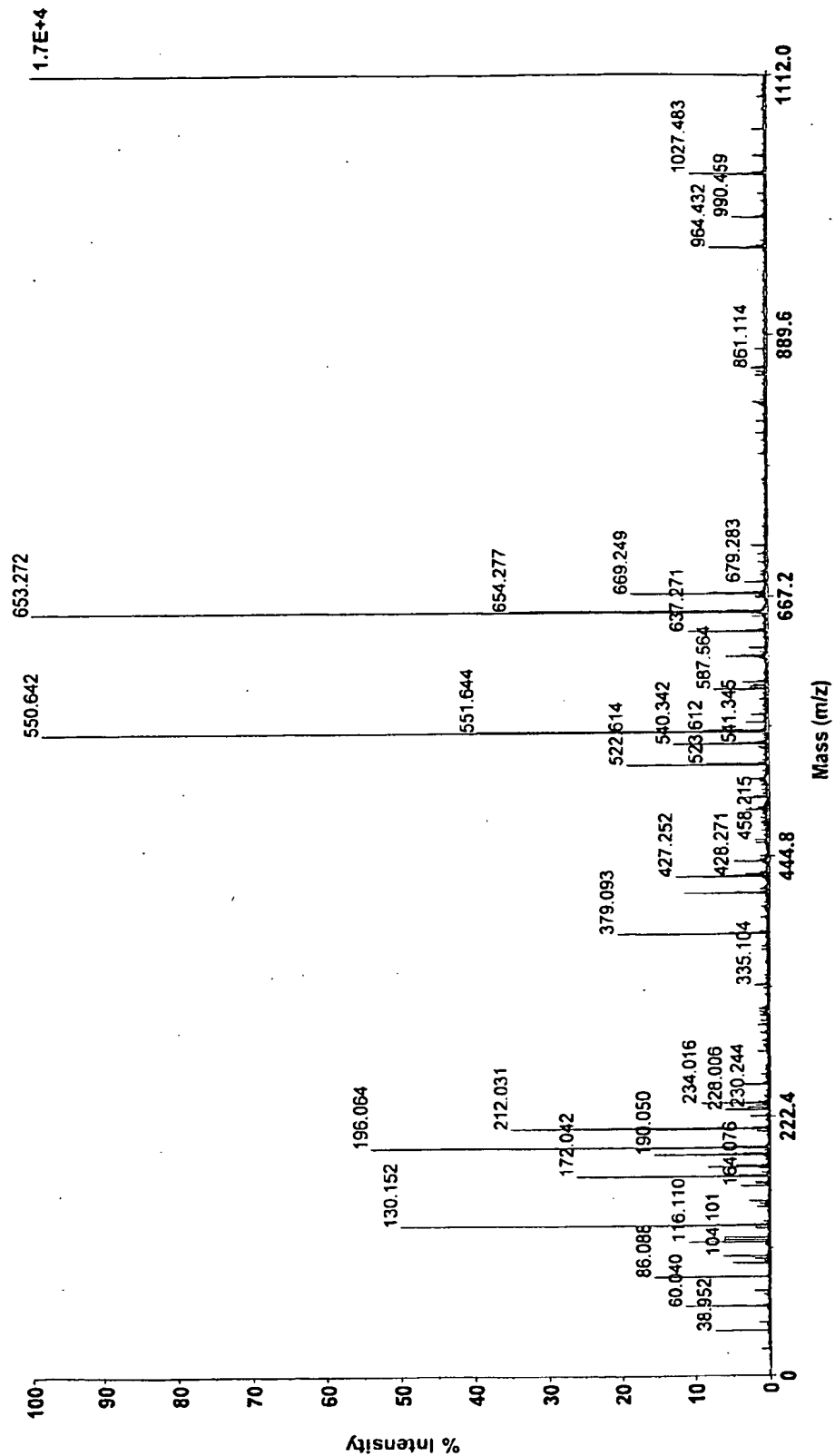
Calibrated MALDI-TOF MS spectrum showing ions formed from photopatterned *N*-Tris(2,4,6-trimethoxyphenyl)phosphine-GGFL ( $m/z=964.4$  Da) peptide. Inset image of photopatterned array of Texas Red sulfonylethylphosphine (Red) and fluorescein isothiocyanate (green). *N*-Tris(2,4,6-trimethoxyphenyl)phosphine (TMPP) is facilitates product detection and formation of a ions for post source decay analysis. Analytical Biochemistry 268, 305-317 (1999).



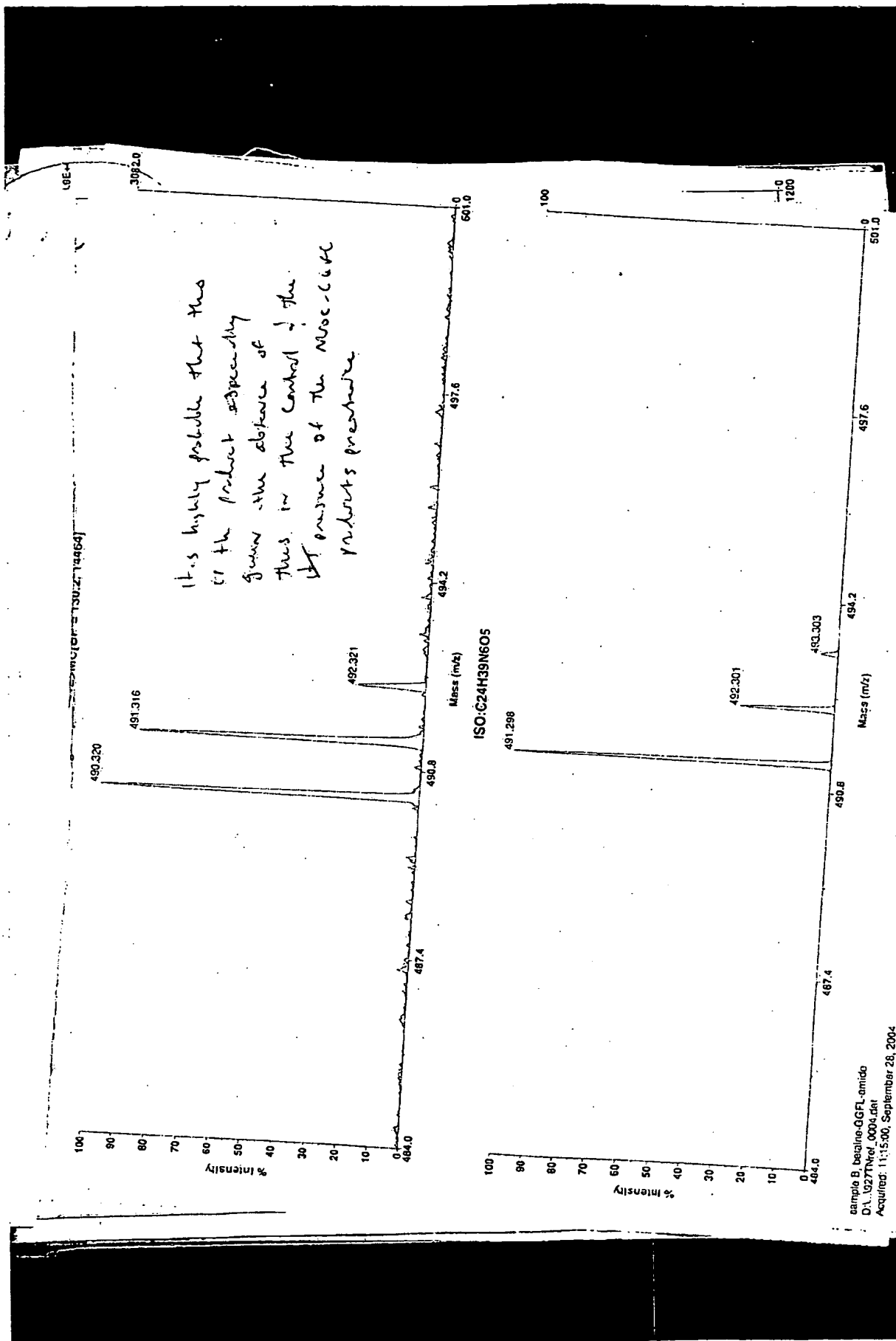
Calibrated MALDI-TOF MS spectrum of observed isotopic distribution for the  $m/z=964.4$  Da ion vs. those predicted for the TMPP-GGFL [C48H63N5O14P] (bottom).

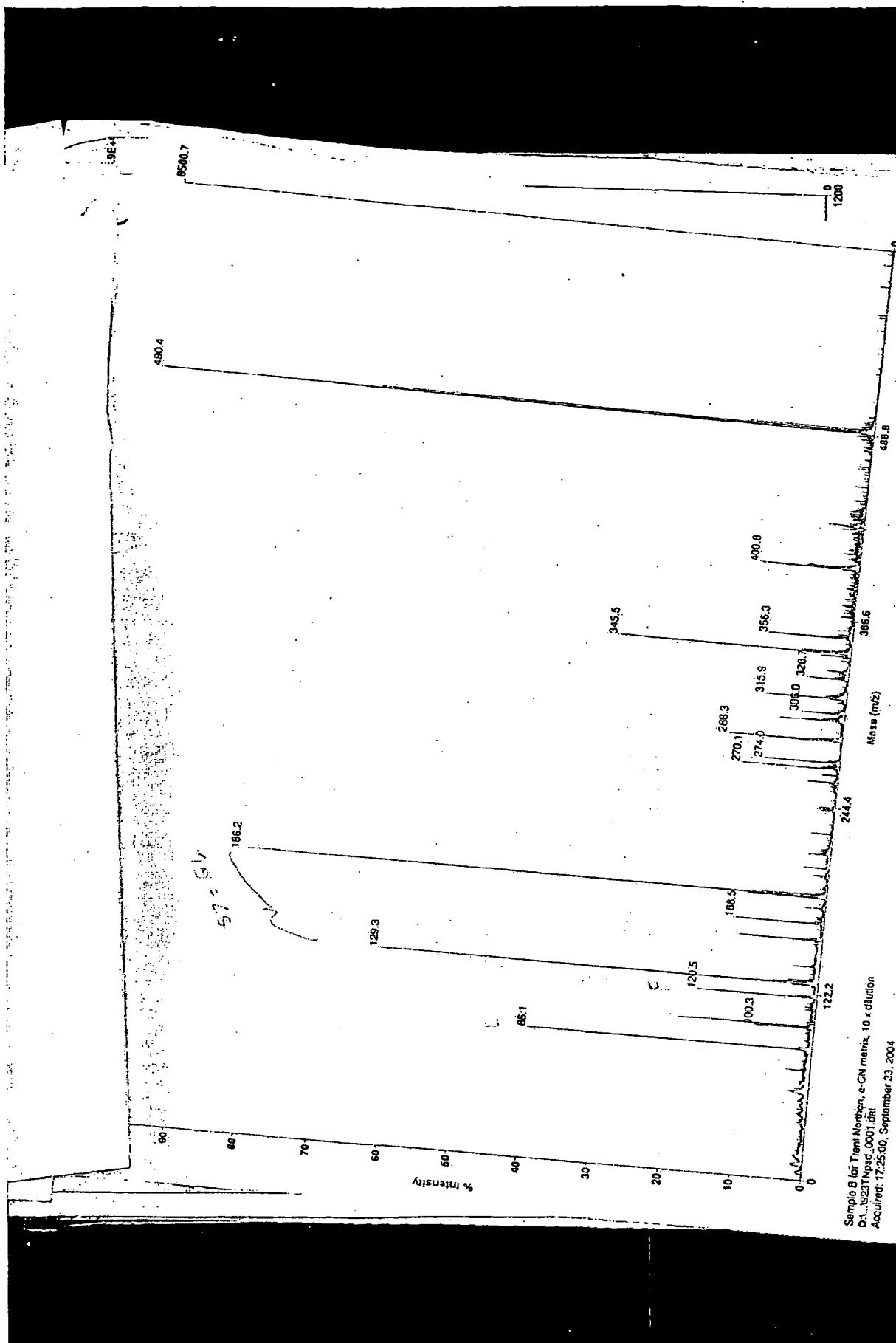


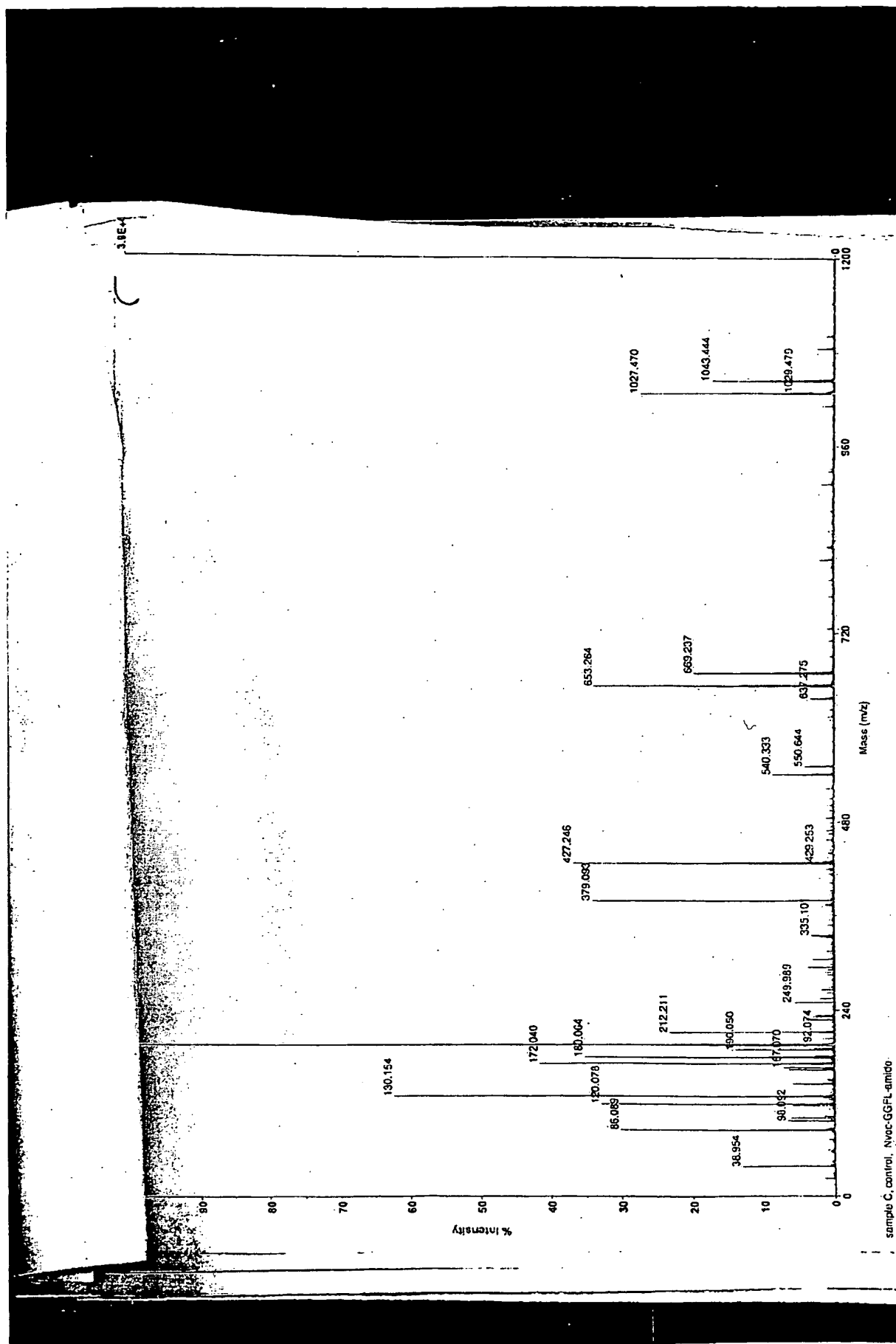
Uncalibrated MALDI-TOF MS post source decay showing the a1 (TMPP-G), a2 (TMPP-GG), a3 (TMPP-GGF), and primary ion m/z=964.8 Da of the TMPP-GGFL peptide.



Calibrated MALDI-TOF MS spectrum showing ions formed from control (not irradiated) areas.







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From the INTERNATIONAL BUREAU

**PCT**NOTIFICATION CONCERNING  
SUBMISSION OR TRANSMITTAL  
OF PRIORITY DOCUMENT

To:

ATKINS, Robert, D.  
Quarles & Brady Streich Lang, LLP  
One Renaissance Square  
Two North Central Avenue  
Phoenix, AZ 85004  
ETATS-UNIS D'AMERIQUE

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 19 July 2005 (19.07.2005)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 112624.00138 PCT	
International application No. PCT/US2005/015764	International filing date (day/month/year) 06 May 2005 (06.05.2005)
International publication date (day/month/year)	Priority date (day/month/year) 06 May 2004 (06.05.2004)
Applicant ARIZONA BOARD OF REGENTS, acting for and on behalf of, Arizona State University et al	

- By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
- (If applicable) The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
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<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
06 May 2004 (06.05.2004)	60/569,370	US	20 June 2005 (20.06.2005)
10 September 2004 (10.09.2004)	60/608,774	US	20 June 2005 (20.06.2005)
29 October 2004 (29.10.2004)	60/623,181	US	20 June 2005 (20.06.2005)

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